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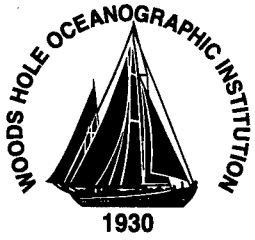

DOCTORAL DISSERTATION

The Aggregation of Clay Minerals and Marine Microalgal Cells: Physicochemical Theory and Implications for Controlling Harmful Algal Blooms

by

Mario Rhuel Sengco

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Physicochemical Theory and Implications for Controlling Harmful Algal Blooms

By

Mario Rhuel Sengco

B.S., Southampton College, Long Island University, 1994

Submitted in partial fulfillment of the requirement for the degree of

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ABSTRACT

In recent years, the use of clay minerals has emerged as one of the most promising strategies for directly controlling harmful algal blooms (HABs). Its principle is based on the mutual aggregation of algal cells and mineral particles, leading to the formation of large flocs that rapidly settle to the ocean floor. This work investigated the effectiveness of various domestic clays against a number of bloom-forming species from the United States. Twenty-five clays were tested against the dinoflagellate, *Karenia brevis* (formerly *Gymnodinium breve*), and the chrysophyte, *Aureococcus anophagefferens*. In general, the highest removal efficiencies (RE > 90% at 0.25 g l^{-1} of clay) against *K. brevis* were found using montmorillonite, bentonite and phosphatic clays (i.e. a product of phosphate mining containing large amounts of montmorillonite). The RE of phosphatic clays remained high (> 80%) even at 0.03 g l^{-1}. Kaolinite and zeolite were mostly ineffective against *K. brevis*. Removal with clay exceeded those for alum, polyaluminum chloride (PAC) and several other polymeric flocculants by a factor of two. However, the combination of phosphatic clay and PAC (at 5 mg l^{-1}) decreased the amount of clay needed to maintain 80% RE by one order of magnitude. Cell viability and recovery remained high when clay loading stayed below 0.03 g l^{-1} with or without resuspension of the sediment. However, cell mortality approached 100% with 0.50 g l^{-1} even with daily resuspension. Between 0.10 and 0.25 g l^{-1}, *K. brevis* survival and recovery depended on the interplay of clay loading, the frequency of resuspension, and duration of contact prior to the first resuspension event. For *A. anophagefferens*, the RE did not exceed 40% for any clay at 0.25 g l^{-1} even in combination with coagulants and flocculants. The highest removal was achieved by thoroughly mixing the clay slurry (e.g. phosphatic clay) into the cell culture.

The RE by phosphatic clay varied significantly in a survey consisting of 17 different species from five algal classes. Moreover, the removal trends varied substantially with increasing cell concentration. For example, cell removal increased with increasing clay loading and cell concentration for *K. brevis*. However, RE dropped below 70% when cell concentration was < 1000 cell ml^{-1} for clay loadings up to 0.50 g l^{-1}. This suggested that a critical number of organisms should be present for clays to remain effective. Similarly, enhanced removal with increasing cell concentration was also found in *Akashiwo sanguinea* (formerly *Gymnodinium sanguineum*), *Heterosigma akashiwo* and *Heterocapsa triquetra*. In the six remaining species, RE initially increased then decreased, or RE remained constant as more cells were treated. The removal pattern among the species at comparable cell numbers did not correlate with the cross-sectional area (R^2 = 0.23), swimming speed (R^2 = 0.04), or a type of cell covering (i.e. theca, silica frustule).

However, when the total collision frequency coefficients were calculated (including collisions due to cell motility) over the interval when clays were < 50 μm, these values correlated well with the empirical RE's for the flagellated species ($R^2 = 0.90$). These results suggested that collisions due to cell motility may be important during the early stages of aggregation when clay sizes are relatively small (i.e. near the surface where the clay layer is initially added).

The electrophoretic mobility (EPM) of marine microalgae displayed a small range of negative values. While the values were smaller that those reported from freshwater species, these results confirmed earlier assumptions that marine species carry a negative charge like their freshwater counterparts. In addition, these results also revealed that the stabilities of cell suspensions in seawater are not controlled by charge neutralization. However, these measurements did not provide direct information on why one species was more readily removed over another by a given clay mineral (e.g. phosphatic clay).

The EPM of clays in freshwater also exhibited predictable negative values, with montmorillonites showing the highest stability and phosphatic clays the lowest. Kaolinite and zeolite displayed a range of intermediate values. These differences vanished when the clays were suspended in natural seawater (29.6 salinity), reducing the surface charge to a small range of negative values. This effect occurred even at 1/16 of the final salinity (1.85 salinity). Viewed alone, these results did not provide direct information on why one clay mineral worked better than another against a given algal species (e.g. *K. brevis*).

Kinetic and modelling experiments using *K. brevis* and three minerals revealed some distinct patterns in aggregation and settling among the clays, including how they removed the organisms. After dispersing on the surface, phosphatic clays aggregated quickly by virtue of low stability (low EPM). Cell removal coincided with the onset of settling. Also, kaolinite aggregated quickly and was controlled by size as well as stability. However, cell removal followed clay settling over 40 min, after which cell removal decreased yielding only 46% RE. Bentonite aggregated slowly over 90 min due to its high stability (high EPM), but produced a number of large voluminous flocs that steadily removed the algae. The sinking rate of flocs increased as cells became incorporated, but the onset of settling was delayed when cells were present in phosphatic clay and kaolinite due to a predicted reduction in aggregate density. The process of kinetics and sedimentation were modelled using first order equations for all mineral-algae combinations.

Finally, phosphatic clays demonstrated the ability to selectively remove *K. brevis* in a mixed culture with the dinoflagellate, *Prorocentrum micans*, or the diatom, *Skeletonema costatum*. While the RE's were generally comparable to individual cultures, the RE of either species increased in the presence of the other, especially for *K. brevis*. Similar results were observed in mesocosm studies using a natural assemblage during a *Karenia* bloom. In fact, the RE of *K. brevis* were higher than would be predicted from single-species laboratory studies given its low initial concentration.

Overall, this research demonstrated the effectiveness of clay treatment against a number of HAB species in the U.S. This work also provided new insights into the aggregation phenomenon between minerals and living algal cells by focusing on the physical (cell size), chemical and behavioral (i.e. motility) properties of both particle types, the effect of particle concentration, and the aggregation kinetics of the clay-algae system.

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mother, Marina L. Desiderio, thank you for all the love and support - I know you are with me in spirit.

I wish to dedicate this work to the memories of my father, Alfonso Vicencio Sengco, and my grandfather, Soliman Estrella Desiderio, whose sacrifice though the early years have made this possible.

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CHAPTER 1

Introduction

Harmful algal blooms (HABs), commonly known as red tides, are natural aquatic phenomena resulting from the proliferation and accumulation of certain species of micro-algae, many of which have severe impacts on public health, industry, other aquatic organisms, and the quality of freshwater and marine environments. They are a recurrent and costly problem throughout the world, and in recent years, they have presented numerous scientific and management challenges. Efforts to manage HABs have focused mainly on prevention and the amelioration of their impacts. Some of these include pollution reduction, coastal monitoring and forecasting, satellite remote sensing, toxin monitoring and harvest restrictions of contaminated products. While effective, these programs often treat the apparent "symptoms" of HABs without dealing with the causative organisms directly (Anderson, 1997). At present, there are virtually no strategies in place to treat an existing or persistent outbreak that threatens a coastal population and its livelihood.

In the United States, the last attempt to treat a red-tide in the field took place in 1957 when copper sulfate was dispersed along the Gulf coast of Florida (Rounsefell and Evans, 1958). The bloom was temporarily controlled but returned to most locations within days. While much has been learned about the dynamics of the Florida red-tide which can explain the disappointing 1957 results (e.g. transport of established populations from offshore to replace those that were treated), the possible collateral damage due to copper sulfate dispersal may hinder its future practicability. In Japan, a number of control methods have been attempted such as ozonation, ultrasonics, strong bubbling of the water column, skimming the surface to remove the algae, and the use of flocculants (Shirota, 1989). Chemical and biological control have also been investigated by research groups (e.g. viruses, parasites, pathogens and algal grazers), although their application has been limited to laboratory trials thus far (Nishitani et al., 1984; Suttle et al., 1990; Nagasaki et al., 1994)

In recent years, one of the most promising strategies for controlling HABs has been the used of clay minerals (Shirota, 1989; Yu et al., 1994a; Anderson, 1997). This approach is based on the mutual aggregation between the organisms and the mineral particles, leading to the formation of large flocs that settle to the ocean floor (Avnimelech et al, 1982; Degens and Ittekot, 1984). In the process, the algae are physically removed from the water column, and thus mitigating their possible impacts. Clay-cell aggregation

has proven effective in laboratory experiments (Maruyama et al., 1987; Yu et al., 1994a; Na et al., 1996), and in a number of field trials in Japan (Shirota, 1989) and South Korea (Bae et al., 1998; Choi et al., 1998; Choi et al., 1999). Clay minerals such as montmorillonite and clay-bearing material such as yellow loess (i.e. mixture of gibbsite, quartz and kaolinite) have been very effective against the fish-killing dinoflagellate, *Cochlodinium* sp. Cell removal efficiency (RE) exceeded 90% in most cases with virtually no reported mortality in the caged fish due to clay treatment. Water transparency improved to a depth of 4 m within hours of dispersal, which was followed by the recovery of the moribund fish. In freshwater impoundments, the mutual aggregation of algae with clay is a well-known phenomenon which has been used to remove fine suspended sediments from in treatment facilities, settling ponds and reservoirs (Leslie et al., 1984).

Finally, instead of removing HAB organisms, clay minerals have also been used in Australia to absorb excess inorganic nutrients from the water column (e.g. phosphorus) to sequester them the algae (Higgins, 2000). In Japan, clays mixed with lime were deposited to the ocean floor in order to minimize the regeneration of important nutrients that can stimulate an outbreak (Shirota, 1989).

The main objectives of this research were to investigate the effectiveness of domestic clay minerals against bloom-forming species from the United States, and to elucidate the underlying mechanisms involved in the aggregation and settling process based on physicochemical concepts. Much of this work focused on the removal of the Florida red-tide organism *Karenia brevis* (formerly *Gymnodinium breve*) with phosphatic clay, a montmorillonite-rich deposit from central Florida. Before proceeding some additional definitions and concepts need to be presented.

Clay minerals

Generally, the term "clay" has two meanings (Grim 1953). As a particle definition, clays describe any finely-divided, usually inorganic material, in the size range of < 2 μm. In terms of chemical composition and structure, a clay is a hydrous aluminosilicate mineral with varying amounts of iron, alkalies and alkaline earth elements. It consists of sheet-like layers arranged in a crystalline structure (Swartzen-Allen and Matijevic, 1974).

In one layer, silicon molecules are surrounded by four oxygen molecules forming a tetrahedral arrangement (i.e. siloxane sheet). In another layer, aluminum molecules are surrounded by six oxygen and hydroxide ions in an octahedral arrangement (i.e. alumina sheet). Variations in the number, composition and arrangement of these layers produce the myriad types classified by Millot (1970). Clay minerals also vary in shape, ranging from flat sheets, rods, flakes, to a few amorphous types. Clay minerals are produced from alterations of aluminum silicate feldspars through weathering and low-temperature hydrothermal processes (Knauskopf, 1967). In natural settings, clays are a common and abundant constituent of soils and marine sediments. They are transported to the aquatic environment by hydrothermal activity, terrestrial run-off (Milliman and Meade, 1983; Smith and DeMaster, 1996) and wind (aeolian) transport (Ittekkot, 1993).

Clay minerals were selected for the purpose of treating HABs for their effectiveness at removing the causative organisms based on the results from Asia. They have displayed a wide range of affinities for both freshwater and marine species in culture. Moreover, clays are natural solids that are considered a low risk for causing environmental damage (Portman, 1970; Howell and Shelton, 1970; McIntyre, 1983, Shirota, 1989). From a practical standpoint, clays are relatively inexpensive, available in large quantities and easy to prepare.

The two prominent clay minerals used in removal studies are montmorillonite and kaolinite. Montmorillonite is a three-layered clay composed of an alumina sheet between two siloxane sheets. It displays a strong propensity for exchanging ions in its crystal structure. It also adsorbs water readily into spaces between the layers, thus allowing it to swell to almost double its size. Kaolinite is a two-layered clay made up of one siloxane and one alumina sheet. Typically, it has a low ion-exchange capability per unit mass and no swelling capacity.

In previous reports, the term clay has been used to refer to samples that coincided with the size definition (i.e. < 2 μm fraction), although its actual mineral composition had both clay (i.e. aluminosilicate) and non-clay particles. One example is yellow loess from South Korea which contained aluminosilicate (e.g. kaolinite) and non-clay minerals such as quartz and gibbsite. While the reported effectiveness of this composite sample was >90% against the target species, there was no effort made to ascertain whether a specific

mineral component of loess may be responsible for cell removal. Therefore, it may be questioned whether the aluminosilicates were important at all in cell removal in this case. For this thesis, the term clay will be used to refer specifically to aluminosilicate minerals, such as kaolinite and montmorillonite, that also meet the size definition. While early experiments will be conducted using pure aluminosilicates, composite samples that contain both aluminosilicate and non-aluminosilicate minerals will be scrutinized further in order to determine whether a certain fraction is responsible for cell removal.

Physicochemical aggregation

The chief process involved in clay-algae removal is aggregation. According to physicochemical theory, aggregation can be divided into two sequential steps (O'Melia and Tiller; 1993; Elimelech et al., 1995): transport and attachment. Transport is a physical process that generates particle collisions and is controlled by the hydrodynamics of the system and external forces such as gravity. The three main mechanisms are Brownian diffusion, fluid motion (laminar or turbulent), and differential sedimentation. In diffusion, collisions are generated by the random motion of particles due to thermal effects. In fluid motion, collisions occur as two particles travelling at different velocities in the flow stream make contact. During differential sedimentation, collisions take place as a larger, more-rapidly sinking particle intercepts a smaller, slower particle. McCave (1984) determined that certain mechanisms become dominant during specific intervals of particle size: Brownian diffusion is important when particles are < 1 μm, while fluid motion begins to dominate for larger particles, depending on the shear rate. Differential sedimentation is more important for the largest particles, until they are lost to the system by settling. Similarly, Hunt (1980) identified such regions in the particle spectrum and developed a model using a power law function to calculate the influence of each mechanism as the particles move through the size distribution. In a system with flagellated organisms, like some of those that produce HABs, particle collisions may also be generated by their swimming ability (Jackson and Lochmann, 1993).

After collision, particle attachment occurs when the particles adhere to each other to produce an aggregate or floc. This process is controlled by the surface chemical properties of the particles and chemical properties of the surrounding medium (e.g. pH,

ionic strength, valence of the ions, polyelectrolytes). Clay minerals develop surface charge through the exchange of ions of lower valence in the crystal structure (i.e. isomorphic substitution), the specific adsorption of charged species from the medium, and the exchange of ions along the mineral surface. These charges, typically electronegative, are balanced by excess ions (i.e. counterions) from the medium to form the so-called double layer arrangement (Thomas et al., 1999). The interaction of similarly-charged double layers leads to electrostatic repulsion and the poor attachment of colliding particles (i.e. stable suspension). As the concentration of counterions increases, the double layer is compressed, allowing attractive forces (e.g. van der Waals) to dominate, and attachment to occur. Particle attachment may also take place through polymer bridging, a process that involves the adsorption of a long-chained molecules between two charged particles (Gregory, 1987).

Freshwater algae have been found to have negative surface charges (Ives, 1956; Geissler, 1958). These charges arises from the ionization of functional groups on the various organic molecules on the cell surface (e.g. amino acids, nucleic acids, proteins, lipids, and carbohydrates). Similar charge measurements for marine microalgae are lacking, although the same charge nature has been has been assumed (Maruyama et al., 1987; Yu et al., 1994a). In seawater, the thickness of the double-layer is small (Stumm and Morgan, 1996) and the stability of the cell suspension may be controlled by organic polymers (i.e. steric stabilization) (O'Melia and Tiller, 1993).

In studies of bloom aggregation in the laboratory, Kiorboe et al. (1990) parameterized the algal stickiness coefficients (α) for several species. The term α is the ratio of the number of collisions leading to successful attachment to the total number of particle collisions. Generally, it is inversely related to the stability of the suspension. They found that diatoms, as a group, were stickier than dinoflagellates. Within that group, stickiness also varied among species and changed over time for some individual species: some increased stickiness as the culture aged, while others displayed a contant level throughout the growth cycle.

Clay-algae aggregation

The aggregation suspension composed of clay minerals and algal cells is complex. It consists of different-sized particles (i.e. heterodisperse) ranging from submicron (clays) to tens of microns for the largest algal species. Moroever, the particles have varying shapes, composition (i.e. inorganic vs. organic), surface properties, and behavior (i.e. non-motile vs. motile). Nevertheless, the same physicochemical concepts have been applied to the clay-algae system as used in clay-clay or cell-cell aggregation, although in a mostly qualitative and descriptive manner (Avnimelech et al., 1982; Alldredge and Silver, 1988; Shirota, 1989). In the current model, transport and collisions are brought about by the same suite of mechanisms (Alldredge and Silver, 1988). However, Brownian diffusion has often been excluded since its effectiveness diminishes in the size range of most algal species (Yu et al., 1995b). Avnimelech et al. (1982) and Leslie et al. (1982) proposed that the attachment of clay particles on the cell surface was mediated by surface-active organic polymers produced by the organisms. In essence, the clays (specific gravity about 2.6) act as mineral ballast for the cells. Using theoretical calculations, Yu et al. (1994a) determined the surface repulsive forces between the particles in order to explain the binding affinity of two different clays on the organism surface over a range of pH values.

In most reports, montmorillonites have shown the greatest versatility against a wide variety of algal species (Avnimelech et al., 1982; Maruyama et al., 1987, Soballe and Threlkeld, 1988; Na et al., 1996). Shirota (1989) attributed this ability to the high adsorptive (ion-exchange) capacity of this three-layered mineral. By contrast, Yu et al. (1995a) found that kaolinites were better overall than montmorillonites against several species. Based on theoretical calculations of surface repulsive forces, they explained that the surface properties of kaolinites were more suitable for attaching onto the cell surface than montmorillonite. This issue remains unresolved. Yu et al. (1994a) examined the possible effect of clay size by using diffusion equations and showed that "collision probability" approached a minimum as clay size and cell size became equal. However, questions remain whether these equation were appropriate for larger particles (McCave, 1984). Nevertheless, the prevailing hypothesis is that clay surface charge, dictated by mineralogy, is more important in determining removal ability for a given species.

Focusing on algal properties, Yu et al. (1994b) found that two diatom species, *Nitzschia pungens* and *Skeletonema costatum*, were removed more readily than two dinoflagellates, *Prorocentrum minimum* and *Noctiluca scintillans*, by both kaolinite and montmorillonite. The authors explained that the higher removal of diatoms may be attributed to a higher "specific surface area" associated with their size and shape. The effect of cell concentration was not addressed. In addition, the higher removal of diatoms was linked to stronger adsorption of clays to the cell surface due to the higher amounts of organic matter which are typically associated with this group of marine organisms. With regards to the impacts of clay treatment, the mutual aggregation and sedimentation of certain algae with clays can lead to the preferential removal of co-flocculating species to the sediment, while the resistant species may become enriched at the surface (Avnimelech et al., 1982). Hence, species composition and relative abundance of groups will be affected (Soballe and Threlkeld, 1988). While there have been numerous studies of clay removal using individual species, there have been no reports of clay treatment of water masses containing a mixture of algal species. Cell mortality has also been reported following clay treatment (Shirota, 1989; Bae et al., 1998), although the exact mechanism for cell death is not fully understood.

Finally, the kinetics of clay-cell aggregation remain poorly understood. Several workers have used a second order model to describe the mass flux of heterodisperse suspensions in seawater (e.g. Hunt and Pandya, 1984). Indeed, Yu et al. (1995b) described the process as a second-order reaction in which clay-clay aggregation preceeded clay-cell aggregation. However, it was unclear how the authors differentiated between the loss of clays and and the loss of algae. Moreover, the authors did not include particle settling in their model, leading to an overestimation of the rate constant in their equations.

Research objectives and thesis chapters

The overall objectives of this thesis were to determine the effectiveness of domestic clays at removing a number of bloom-forming species from the United States, to study the effect of clay treatment on algal viability and growth and to explore the effect of clay dispersal on the composition of the phytoplanktonic community over short time scales. Another objective of this thesis was to understand the underlying mechanism of mineral-

algae aggregation and sedimentation by studying the effect of particle concentration, the physical, chemical and behavioral properties of the particles, and the kinetics of aggregation in the heterodisperse system.

In Chapter 2, the effectiveness of various domestic clays was determined against bloom organisms prevalent in the United States, and the effects of clay treatment on viability and cell growth were explored. Laboratory experiments focused on two marine species: the dinoflagellate, *Karenia brevis* (= *Gymnodinium breve*), and the small chrysophyte, *Aureococcus anophagefferens*. Clay removal efficiency (RE) was also compared to those of conventional coagulants (e.g. alum, polyaluminum chloride), a host of polymeric flocculants (e.g. cationic, anionic, non-ionic), and to the RE of clays treated with these chemicals. To study the impacts of clay on the organisms, tests were conducted on the viability and subsequent recovery of cells following treatment and the resuspension of the aggregates.

In Chapter 3, the effects of algal size, motility and con-centration on removal were investigated using a phosphatic clay. Several marine species with varying phy-sical features and swimming speeds were tested. The comparison also included the theoretical collision frequency coefficients for each organism as they collided with a range of clay sizes. Collision coefficients were also calculated for cell motility. In the second part of the chapter, the removal efficiency of *Karenia brevis* was determined in the presence of two other phytoplankton species, in order to determine whether selective removal was possible. A similar study was performed in mesocosms using a natural assemblage collected during a bloom of *Karenia brevis*.

In Chapter 4, the electrophoretic mobilities (EPM) of marine microalgae and clay minerals suspended in a range of salinities were determined. The objectives were to characterize and measure the surface charge, and to relate these properties to the observed removal patterns from previous empirical work.

In Chapter 5, the kinetics of aggregation and settling in the clay-algae system were explored. This investigation focused on *K. brevis* and three clay minerals with varying removal abilities. The objectives were to describe the physical process of aggregation and settling, and to develop a mathematical expression for each system.

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CHAPTER 2

Removal of red- and brown-tide cells using clay flocculation. I. Laboratory culture experiments with *Gymnodinium breve* and *Aureococcus anophagefferens*

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Abstract

Twenty-five domestic clays and Loess clay from South Korea were tested for their ability to flocculate and remove cells of *Gymnodinium breve* (the Florida red-tide organism) and *Aureococcus anophagefferens* (the New York brown-tide organism). Twelve clay samples, consisting mostly of montmorillonite, bentonite and Florida phosphatic clay displayed removal efficiencies greater than 90% against *G. breve* at a clay loading of 0.25 g l^{-1}. Further tests with IMC-P2 phosphatic clay indicated that removal rates can reach as high as 80% at 0.04 g l^{-1}. By contrast, the removal values at 0.25 g l^{-1} against *A. anophagefferens* did not exceed 40% for all clays, but increased to 80% when the clay was dispersed throughout the culture at the time of addition. The removal efficiency of aluminum sulfate (alum), polyaluminum chloride (PAC), and 4 organic flocculants were significantly lower than clays against both organisms (30% to 50%). However, the addition of 5 ppm PAC lowered the amount of clay needed for removal of *G. breve* by 1 order of magnitude at low clay concentrations. *G. breve* fully recovered and remained viable at clay loadings below 0.03 g l^{-1}, with or without resuspension of the flocs, although their recovery and subsequent growth were delayed by 24 h compared to untreated cells. High cell mortality (up to 100%) and no recovery were observed at clay amounts of ≥0.50 g l^{-1} even with daily resuspension of the clay/cell pellet. At intermediate clay loadings (e.g. 0.10 to 0.25 g l^{-1}), survival and recovery depended on several factors: clay amount, the frequency of resuspension, or the duration of contact between the cells and clays prior to the first resuspension event. Regardless of clay loading, cell mortality was extremely low (near zero) after 2.5 h of contact, but increased significantly after 12 h. Preliminary data suggest that cell death may be caused by direct physical contact between the cells and clays and not by the release of potentially cytotoxic substances from the clays or from the lysed cells. Overall, these results show that clays differ substantially in their removal efficiencies, that individual clays differ in their ability to remove different algal species, that flocculants such as PAC can significantly improve clay removal efficiencies, and that the flocculation process can also lead to cell mortality.

Introduction

Over the past 2 decades, the management of harmful algal blooms (HABs) has focused primarily on the amelioration of their impacts. Current strategies include monitoring of HAB cells and toxins, satellite remote-sensing, harvesting and sales restrictions for contaminated products, and sometimes the towing of aquaculture pens away from blooms. While these and other programs have enhanced our ability to protect public health and affected resources, there are virtually no strategies in place to limit the proliferation of the causative organisms, to prevent an imminent outbreak, or to reduce the threat from an existing bloom. In the US, the most recent attempt to control a natural HAB took place in 1957 when copper sulfate was dispersed by planes over 41.5 km^2, stretching along 51.5 km of the Florida coast, to eradicate massive blooms of *Gymnodinium breve* (Rounsefell and Evans, 1958). While the strategy reduced the bloom from several locations, toxicity recurred several weeks later. We now know that the recurrence was due to the movement of new bloom patches to shore from an offshore 'source' region. The study concluded that the copper treatment would only give short-term relief from the red-tide/HAB problem. While this negative assessment is valid, such a result (e.g. several weeks without red-tide impacts) may be a desirable outcome in some circumstances, such as in a public health or ecosystem emergency. Important questions that remain about the success or failure of the copper treatment involve the unknown 'collateral' damage to co-occurring organisms. Copper is lethal to many different organisms, so it is highly likely that this treatment caused ecosystem damage that was not assessed.

In the years after this study, direct control strategies including biological, chemical and mechanical bloom control were pursued by only a few investigators (reviewed in Anderson, 1997) with the exception of investigators in Japan who embarked on an extensive series of mitigation studies. Shirota (1989a) summarizes these efforts, many of which were quickly abandoned, but several of which showed promise. One of the most promising investigated the use of clay to control a bloom of *Cochlodinium* sp. which threatened commercially-valuable finfish in aquaculture (Shirota 1989b). A slurry of seawater and montmorillonite was sprayed into and around several fish enclosures to intercept the bloom. Shortly after treatment, the number of red-tide cells at the surface

was greatly reduced, water transparency increased, and a marked recovery in the reared opaleye and yellowtail was observed.

In a similar effort, clays were used to treat massive blooms of *Cochlodinium polykrikoides* in South Korea (Na et al., 1996). In 1996, approximately 60,000 t of dry Loess clay (kaolinite type) was dispersed by barges over 260 km^2 at a loading rate of 400 g m^{-2}. Removal rates of *C. polykrikoides* were calculated at 90 to 99% up to 2 m depth. No mortality in the aquacultured fish species was reported, and the bloom did not return for the remainder of the season. Consequently, Loess clay has been used in subsequent years to control outbreaks along the southern coast of Korea, with some improvements in the methods of clay preparation and dispersal.

The ability of clays to remove algal cells from suspension is based on the concepts of mutual flocculation and sedimentation. Clay minerals dispersed on surface seawater are quickly destabilized due to its high ionic strength. Destabilization is a process by which the repulsive forces on the clay surface are neutralized by an excess of counter-ions. As electrostatic repulsion decreases, attractive forces between particles dominate (e.g. van der Waals forces), and flocculation occurs when the clay particles collide and coalesce to form larger particles (or flocs). The descending flocs then interact with cells, which either flocculate with the clays or are captured as the flocs sweep through the medium. More detailed studies on the possible mechanisms of clay-cell flocculation have been pursued by several workers (Avnimelech et al., 1982; Soballe and Threlkeld, 1988; Yu et al., 1994a, 1994b).

Given the results in Asia, the use of clays to eradicate an exisiting HAB is a promising and attractive direct-control option for locations with persistent HAB problems. Aside from their effectiveness, clays are appropriate for this purpose because they are generally inexpensive, readily available in large quantities, and easy to use in field operations. In addition, clays are thought to be substances with little or no direct toxic effects on aquatic organisms (e.g. Howell and Shelton, 1970; Portman, 1970; McIntyre, 1983). Moreover, marine organisms are most probably adapted to varying amounts of clay minerals in their environment since clays are a natural and common constituent in river run-off or resuspended bottom sediment. Unfortunately, there were no studies on the environmental impacts of clays in the Asian trials to confirm or reject

these assertions. Efforts are underway in South Korea (H. G. Kim pers. comm.) and in our laboratory (authors' unpubl. data) to focus on the impacts of clays on planktonic and benthic communities and on the surrounding water chemistry.

The purpose of this study was to determine the removal efficiency of various domestic clays against two HAB species from the USA (*Gymnodinium breve* and *Aureococcus anophagefferens*), and to conduct further studies using the most effective minerals. *G. breve* is a naked dinoflagellate responsible for the recurrent blooms along the west coast of Florida and Texas, and occasional outbreaks in North Carolina, Louisiana and Mississippi (Tester and Steidinger, 1997). The cells produce brevetoxin, the potent neurotoxin which kills fish and marine mammals (e.g. manatees; Landsberg and Steidinger, 1998), contaminates shellfish, and causes respiratory problems in humans as a result of toxins in seaspray. *A. anophagefferens* is a tiny, non-motile chrysophyte that forms the 'brown tides' in the bays of eastern Long Island (New York), Narragansett Bay (Rhode Island) and Barnegat Bay (New Jersey) (Cosper et al., 1990). Due to their extremely high biomass (up to 1 x 10^9 cells ml^{-1}), these blooms have major impacts on Long Island ecosystems and have decimated the bay scallop industry since the mid-1980s.

In addition, the removal efficiency of clays was compared to that of chemical coagulants and flocculants which are commonly used in water treatment to remove fine suspended material. Additional tests were conducted to determine if further enhancement of HAB removal would result from combining clays with coagulants and surfactants and through agitation of the clay-algae suspension. Finally, the effect of clay treatment on cell growth and viability of *Gymnodinium breve* was studied using vital stains and culture experiments.

Materials and Methods

Cultures. *Gymnodinium breve* (Wilson strain, CCMP718) and *Aureococcus anophagefferens* (Clone BP3B, provided by E. Cosper) were grown in batch cultures using modified f/2-Si medium under conditions described by Anderson et al. (1999). Growth was monitored using in vivo cellular fluorescence (Model 10-AU Fluorometer, Turner Designs, Sunnyvale, California, USA) calibrated against direct microscope cell

counts (Avnimelech et al., 1982). Removal experiments were performed using cultures in early to mid-exponential growth.

Clay samples and preparation. Twenty-five clay samples were obtained from various US producers and mining operations; Loess clay was obtained from the G.S. Corporation, Seoul, South Korea (Table 2-1). Most samples were provided as fine mineral powders which were used directly in removal experiments. Southwestern montmorillonite (SW-M), Golden Cat Sized Product (GC-SP) and Golden Cat Dyer Mill (GC-DM) were ground by hand with a mortar and pestle, and then sieved to obtain powder between 20 and 74 μm. For the removal experiments, a clay slurry was prepared by suspending a known mass of clay powder in distilled/deionized (DI) water. Freshwater was chosen over seawater as a carrier to minimize the premature flocculation of the clay slurry by salt ions. Three phosphate mining companies from central Florida provided 5 samples of 'phosphatic clay' dispersed in freshwater containing between 3 and 42% solids (clay-sized and non-clay sized particles) (Table 2-1). The percent solid content of the slurry was determined by drying a known mass of wet clay overnight in a laboratory oven (80°C), then dividing the dry weight by the wet weight. Phosphatic clay suspensions for experiments were prepared by diluting the stock solution to the desired concentration using distilled/DI water.

Clay screening and comparison. Ten milliliters of cell culture was placed in triplicate borosilicate test tubes (14 mm inner diam.) The initial cell concentration was determined from in vivo cell fluorescence, and concentrations of 7000 to 10,000 cells ml^{-1} for *Gymnodinium breve* and 3×10^6 to 5×10^6 cells ml^{-1} of *Aureococcus anophagefferens* were routinely used. One milliliter of clay slurry was added dropwise to the surface of the cell suspension using an air-displacement pipet (11 ml final volume). In this set of experiments, the final clay loadings were 0.25, 0.5, 1.0, 2.0, and 4.0 g l^{-1}. One milliliter of distilled/DI water was added to the controls. The clay-cell suspension was allowed to flocculate at 20°C for 2.5 h under quiescent conditions . Afterwards, the supernatant directly above the pellet (here defined as the upper 10 ml) was carefully transferred to a new test tube, mixed, and the number of remaining cells was estimated by fluorescence. The removal efficiency (%RE) was then calculated using the following equation:

Table 2-1. Clay samples tested against *Gymnodinium breve* and *Aureococcus anophagefferens*: 25 domestic clay samples, and Loess clay (South Korea). Clays were grouped according to similarities in removal efficiency based on linkage distances in a cluster analysis (Statistica 5.0, Statsoft Inc, Tulsa, Oklahoma, USA). First 2 columns group clays based on their removal efficiency of *G. breve* (Gymno, G) and *A. anophagefferens* (Aureo, A), respectively (Fig. 2-1).

Gymno	Aureo	Code	Trade Name	Clay mineral type	Company
G1	A3	**MI-BG**	Basco Gel	bentonite	Milwhite, Inc.
G1	A4	**SW-NM**	Nicole Mountain	zeolite	Southwest Mining Group
G1	A3	**WB-B**	Big Horn Bentonite	sodium bentonite	Wyo-Ben, Inc.
G1	A2	**H-DP**	Huber DP-1010	chemically treated kaolin	J.M. Huber Corporation
G1	A3	**CI-200**	Suspengel 200	sodium Western bentonite	CIMBAR performance minerals
G1	A3	**CI-325**	Suspengel 325	sodium Western bentonite	CIMBAR performance minerals
G1	A3	**SP-B**	Spinks Gel Bentonite	Black Hills bentonite	H.C. Spinks Clay Company, Inc.
G1		**IMC-P1**	Phosphate Rock	Smectite mixture,	IMC-Global Operations, Inc.
G1	A3	**IMC-P2**		carbonate-fluorapatite,	IMC-Global Operations, Inc.
G1		**IMC-P3**		palygorskite, mica,	IMC-Global Operations, Inc.
G1		**FIPR**		kaolinite, quartz, wavelite	Florida Institute of Phosphate Res
G1		**NU**		crandellite, dolomite	Nu-Gulf, Mulberry Corporation
G2	A2	**MI-HY**	HY Basco Salt Mud	high yield attapulgite	Milwhite, Inc.
G2	A3	**MI-REV**	Rev-Dust	calcium montmorillonte	Milwhite, Inc.
G2	A3	**SW-M**	Montmorillonite	montmorillonite	Southwest Mining Group
G2	A3	**SW-B**	Bentonite	sodium bentonite	Southwest Mining Group
G3	A3	**H-90**	Polygloss-90	Wrens waterwash kaolin	J.M. Huber Corporation
G3	A3	**GC-DM**	Dryer and Mill Dust	cat litter	Golden Cat, King William, VA
G3	A4	**GC-SP**	Sized Product	cat litter	Golden Cat, King William, VA
G4	A1	**LO**	Loess Clay		South Korea
G4	A3	**MI-K**	Kaolinic	kaolinite and lesser minerals	Milwhite, Inc.
G4	A3	**SP-K**	Ball Clay	Kaolinite	H.C. Spinks Clay Company, Inc.
G4	A3	**SE-CC**	Crown Clay	kaolin	Southeastern Clay Company
G4	A4	**SW-ZP**	Zeo-clino	zeolite	Southwest Mining Group
G4	A4	**SW-NZ**	Natur-Zeo	zeolite	Southwest Mining Group
G4	A3	**H-35**	Huber-35	Huber waterwash kaolin	J.M. Huber Corporation

$$\% \text{ RE} = [1 - (\text{final fluorescence} \div \text{final fluorescence of control})] \times 100 \quad \text{(Eq. 2-1)}$$

The final fluorescence of the control (i.e. 2.5 h after the addition of DI water) was used to account for cell sinking. Removal efficiency was plotted against clay concentration for each clay. To select the most efficient clays for each organism, defined here as the sample displaying the highest removal efficiency at the lowest clay dosage (0.25 g l^{-1}), the initial slope of the removal curve was calculated. The clays were ranked according to this value and they were grouped according to linkage distances using cluster analysis (Statistica 5.0, Statsoft Inc., Tulsa, Oklahoma, USA).

Coagulants and flocculants. In these experiments, the ability of coagulants and flocculants to induce cell flocculation was tested. These chemicals are commonly used to enhance the clarity of drinking water by promoting the rapid flocculation of very fine, slow-settling particles or colloids. Coagulants (e.g. aluminum sulfate [alum] and polyaluminum chloride [PAC]) neutralize the surface charge of particles, reducing the electrostatic repulsion between them to promote their aggregation. By contrast, flocculants (e.g. long-chain organic polymers with reactive ends) function as interparticle bridges, linking particles together which would normally repel one another. Using the screening protocol above, the removal efficiency of 2 coagulants (alum and PAC) and 2 cationic flocculants (Percol LT-7990 and LT-7991) were tested against *Gymnodinium breve*. Also, alum, PAC and 2 flocculants (Percol 720 (nonionic) and Percol 778 (cationic)) were tested against *Aureococcus anophagefferens*. The coagulants/flocculants were prepared using distilled/DI water and added to the cell suspension to a final concentration of 0 (DI water only), 1, 10, 100, 1000 ppm.

Combination of coagulants/flocculants and clays. To determine whether coagulants and/or flocculants can enhance the removal efficiency of clays against *Gymnodinium breve*, increasing amounts of IMC-P2 (phosphatic clay) and SW-NZ (zeolite) were mixed with 1, 10, 100, 1000 ppm of alum, Percol LT-7990 and Percol LT-7991 just prior to the screening protocol. IMC-P2 was also treated with 5 ppm PAC. In the same fashion, H-DP (kaolin), MI-HY (attapulgite) and IMC-P2 were treated with alum, PAC, Percol 720 and Percol 778, and then added to *Aureococcus anophagefferens* cultures.

Pulsed clay addition and agitation experiment. Two variations in the clay screening protocol were performed using *Aureococcus anophagefferens* and selected clays (H-DP, MI-HY and IMC-P2). In the first experiment, the clay slurry was added to each cell suspension in a single pulse (1 ml), 2 pulses (2 x 0.5 ml), 3 pulses (3 x 0.33 ml) and 4 pulses (4 x 0.25 ml) with 15 min between each pulse. The same total amount of clay was added in all cases. The reaction was terminated at 2.5 hours and the final fluorescence measurement was taken as before. In the second experiment, the clays were added in a single pulse, after which the test tube was immediately capped and inverted 3 times to thoroughly disperse the clay and cell suspension. The tube was then placed upright on a rack and flocculation was allowed to proceed undisturbed for 2.5 h. The final cell concentration and removal efficiency were determined as above.

Effect of clay treatment on viability and growth. The effect of IMC-P2 (phosphatic clay) on the viability and growth of *Gymnodinium breve* was studied using 3 strategies, 2 of which consider the role of physical resuspension on the ability of a treated cell to recover. In the first experiment, the clay-cell pellet was incubated in each tube for 2.5, 12, 24 and 48 h at 20°C following clay treatment (0, 0.03, 0.10, 0.50 g l^{-1}). After the remaining cell concentration in the supernatant had been determined (by fluorescence), the supernatant was returned to the original tube and mixed well with the sedimented clay and cells. One milliliter aliquots were treated with 2.5 μM (final) 5-chloromethyl-fluorescein diacetate (CMFDA) (Molecular Probes, Oregon), a vital stain that only penetrates the cell membrane of live cells and reacts with esterases to produce a green signal under FITC fluorescence (450 to 490 nm excitation, Zeiss axioscope). Samples were incubated at 20°C for 20 min in the dark. The first 400 cells encountered on the slide were counted, keeping track of living and dead (dying) cells. Dead cells were identified as intact cells without green FITC fluorescence, or easily discernible cell fragments that contain cytoplasmic material.

In a second experiment, the supernatant was carefully returned to the original tube immediately after the fluorescence measurement without disturbing the pellet. The tubes were incubated at 20°C, and cell growth was monitored over 150 h by making daily fluorescence measurements on the unmixed supernatant. The controls consisted of cultures treated with distilled/DI water (1 ml) or f/2 medium (1 ml).

For the third experiment, the goal was to determine whether potentially viable cells from the pellet can recover and grow if allowed to disaggregate from the pellet as a result of resuspension. After the supernatant had been removed, the clay-cell pellet was resuspended in fresh f/2 medium (10 ml final volume). The volume was distributed equally into 3 new test tubes and incubated for 24 h at 20°C. The cultures were then subjected to a mixing schedule in which the pellet was resuspended daily, every 2 d, or every 3 d. Resuspension was achieved by gently mixing the tube by hand until the material on the bottom was evenly dispersed. Direct fluorescence measurements of the supernatant were taken daily over 7 d using fluorescence prior to each resuspension procedure.

Possible causes of cell mortality. This experiment was conducted to elucidate the possible cause(s) of *Gymnodinium breve* mortality following clay treatment. We tested whether a cytotoxic substance is released into the medium during the clay treatment. After 2.5 h clay treatment, the supernatant was filtered using a syringe filter holder with a Gelman A/E type glass-fiber filter (0.7 μm). The filtrate was enriched with the appropriate amount of f/2 nutrients. Nine milliliters of the filtrate was transferred into test tubes and re-innoculated with an actively growing culture of *G. breve*. Cell growth was monitored over 6 d by fluorescence; 1 ml of DI water was added to control tubes treated in the same fashion.

Other HAB species. The removal ability of IMC-P2 was further tested against 2 additional species following the previous screening protocol: *Alexandrium tamarense* (GTCA 28) and *Heterosigma akashiwo* (CCMP 452).

Results

Clay screening. The removal efficiency of clays against *Gymnodinium breve* generally followed a hyperbolic function with clay concentration (Fig. 2-1A). A cluster analysis based on initial slope of the removal plots revealed that the clays could be placed into 4 groups (Fig. 2-1A, Table 2-1). Twelve clays (Group 1) reached >90% removal efficiency at the lowest concentration tested (0.25 g l^{-1}), after which removal remained constant. This group consisted of all Florida phosphatic clays, several montmorillonites and bentonites, 1 kaolinite and 1 zeolite. Further tests with IMC-P2 phosphatic clay

showed that removal efficiency can reach 80% with as little as 0.04 g l^{-1} (Fig. 2-2). The remaining clays were placed in 3 groups that displayed less efficient removal rates, with Group 4 showing a more linear removal relationship with clay concentration (e.g. Korean Loess clay).

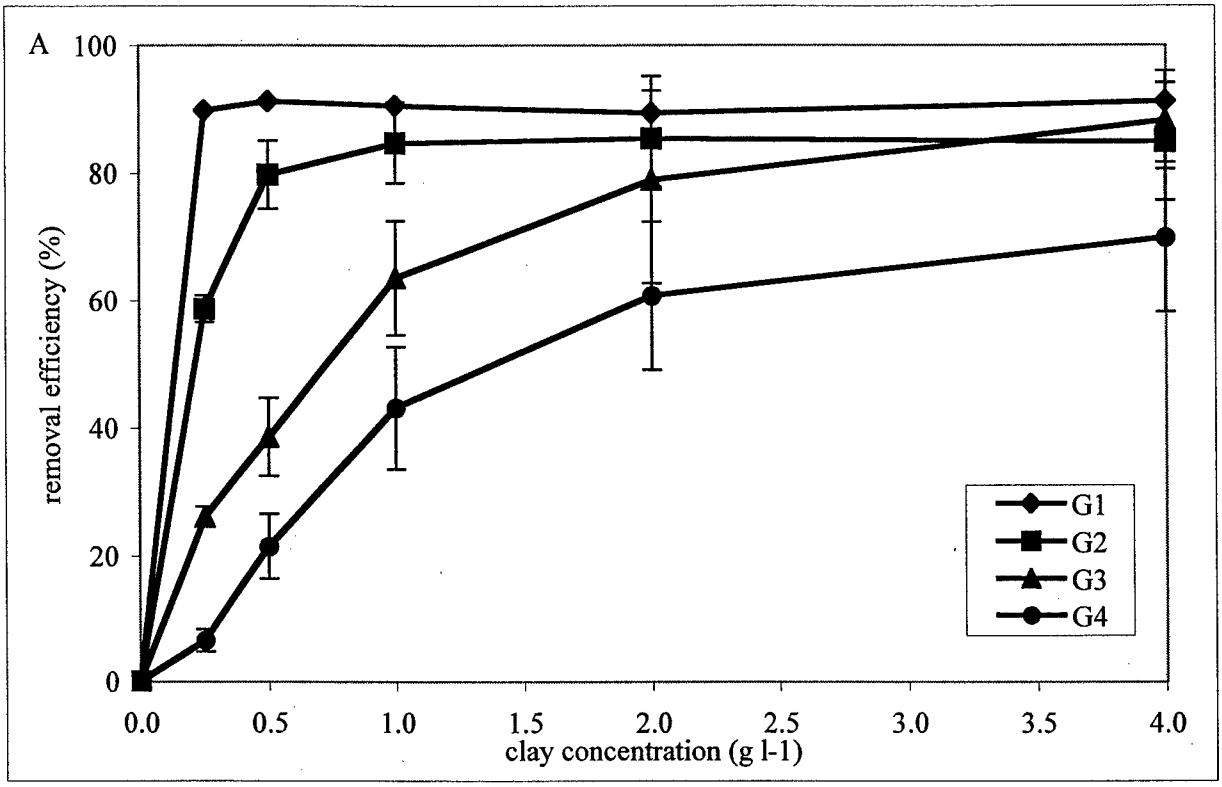
In contrast, the removal efficiency of all clays against *Aureococcus anophagefferens* did not exceed 36% at 0.25 g l^{-1} (Fig. 2-1B). Loess clay (LO), Huber kaolinite (H-DP) and Milwhite attapulgite (MI-HY) displayed the best removal at this concentration. At higher concentrations, removal efficiency increased linearly with increasing clay dosage, and the highest removal observed was 86.6% using 4.0 g l^{-1} (LO, Group 1).

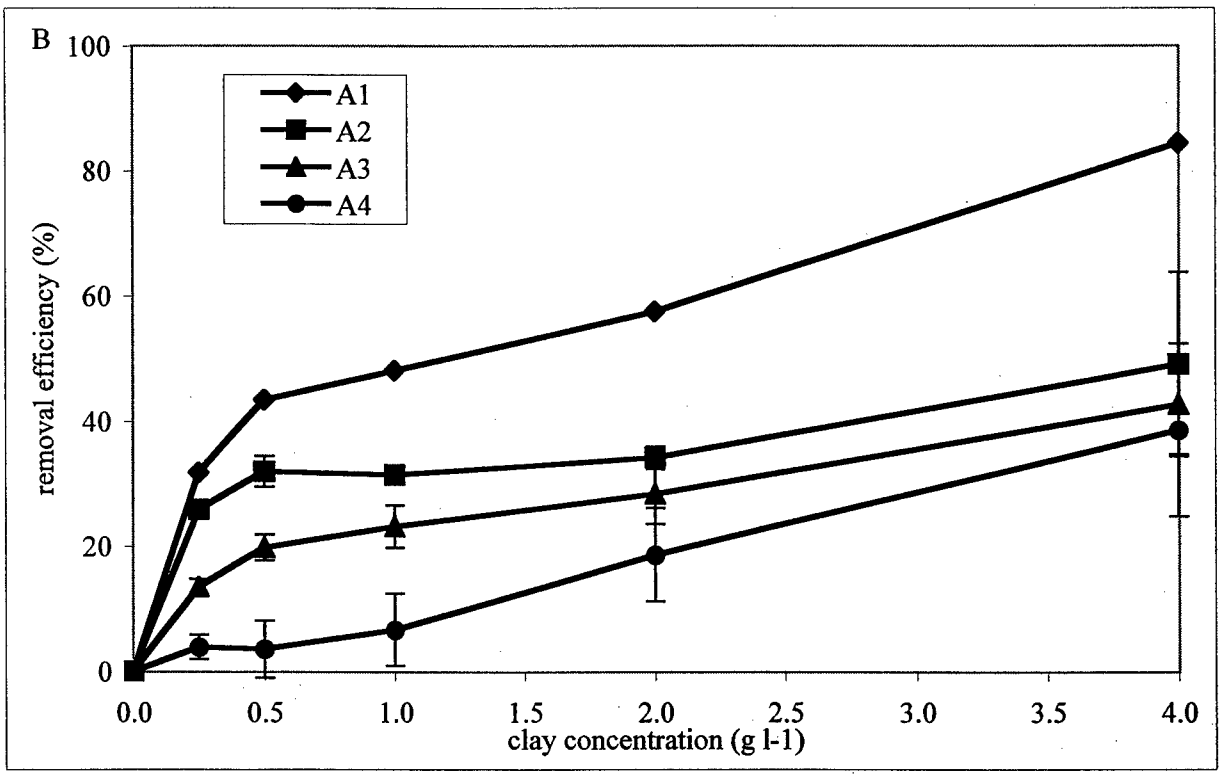
Coagulants and flocculants. The removal efficiency of alum, PAC and both cationic flocculants (Percol LT-7990 and LT-7991) against *Gymnodinium breve* did not exceed 49% within the concentration range tested (Fig. 2-3A). Similarly, alum, PAC and the organic flocculants (Percol 720 and 778) were ineffective against *Aureococcus anophagefferens* with removal rates reaching only 10%. In the case of Percol 778, removal efficiency was negative, which suggested that the flocculant prevented cell sinking relative to the controls (= stabilization). Percol 720 displayed a similar effect between 3 and 10 ppm, but to a lesser extent.

Clays plus coagulants/flocculants. The addition of alum and Percol to IMC-P2 phosphatic clay decreased its removal efficiency of *Gymnodinium breve* (Table 2-2). The addition of >10 ppm Percol LT-7990 and LT-7991 resulted in a decrease in removal efficiency from 89 to 50%, and from 85 to 46%, respectively. The clay suspension had flocculated with itself immediately after addition of the coagulant or flocculant before it could be added to the cultures. Vigorous shaking and vortexing of the clay slurry could not disrupt the particles. After the slurry was added to the culture, clay flocs formed quicker and were much greater in size than untreated clays. These particles sank more rapidly to the bottom of the test tube.

In contrast, the removal efficiency of IMC-P2 phosphatic clay was greatly enhanced by the addition of polyaluminum chloride (PAC) (Fig. 2-2). It took 90% less clay to achieve an 80% removal with the addition of 5 ppm of PAC (cf. 0.01 g l^{-1} clay with and without PAC: Fig. 2-2).

Figure 2-1. Removal efficiency of domestic clays against (A) *Gymnodinium breve* and (B) *Aureococcus anophagefferens*. Clays were grouped by comparing the initial slope of each removal curve (from 0 to 0.25 g l^{-1}). Slopes were analyzed using cluster analysis (Statistica) based on linkage distances. These curves represent the best fit curve for each cluster. G1-G4: clay groupings based on the removal of *G. breve*; A1-A4: clay groupings based on the removal of *A. anophagefferens*. The clays comprising each group are listed in Table 2- 1.


A
100
80
60
40
20
0
removal efficiency (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
clay concentration (g l-1)
G1
G2
G3
G4


B
100
80
60
40
20
0
removal efficiency (%)
0.0
0.5
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3.5
4.0
clay concentration (g l-1)
A1
A2
A3
A4

Figure 2-2. Removal efficiency of IMC-P2 alone and IMC-P2 treated with polyaluminum chloride (PAC) against *Gymnodinium breve*. Lower clay amounts were tested (<0.25 g l^{-1}).

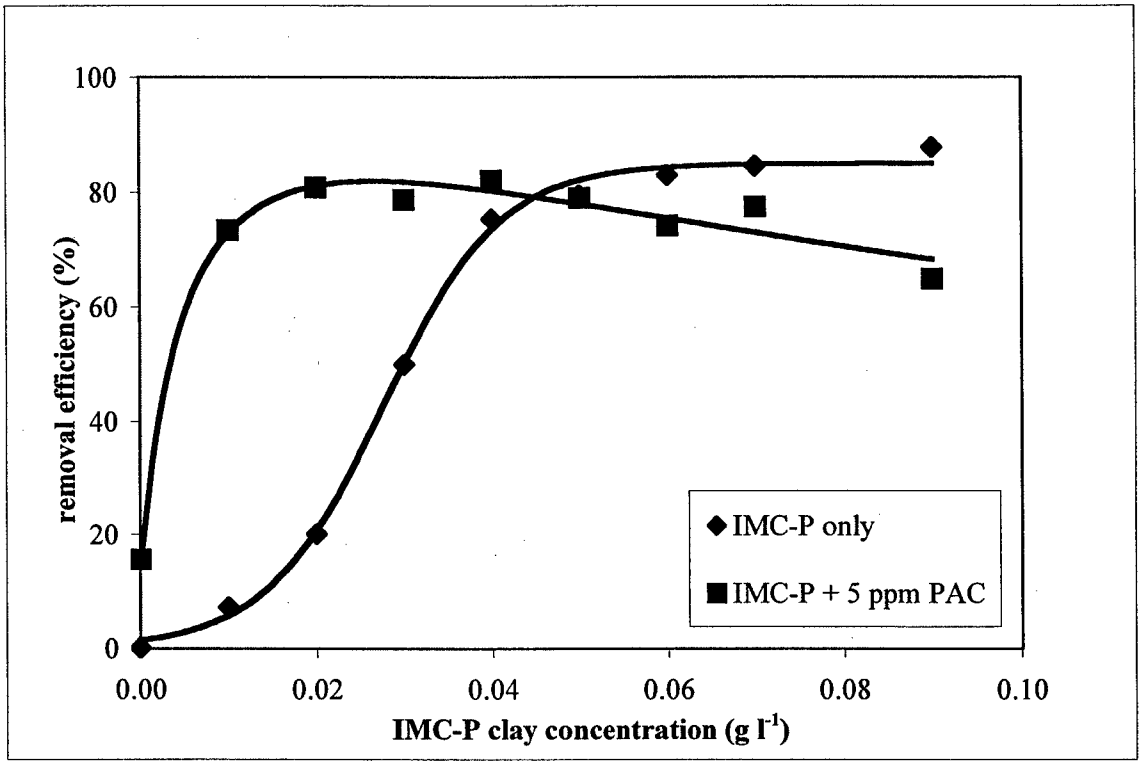
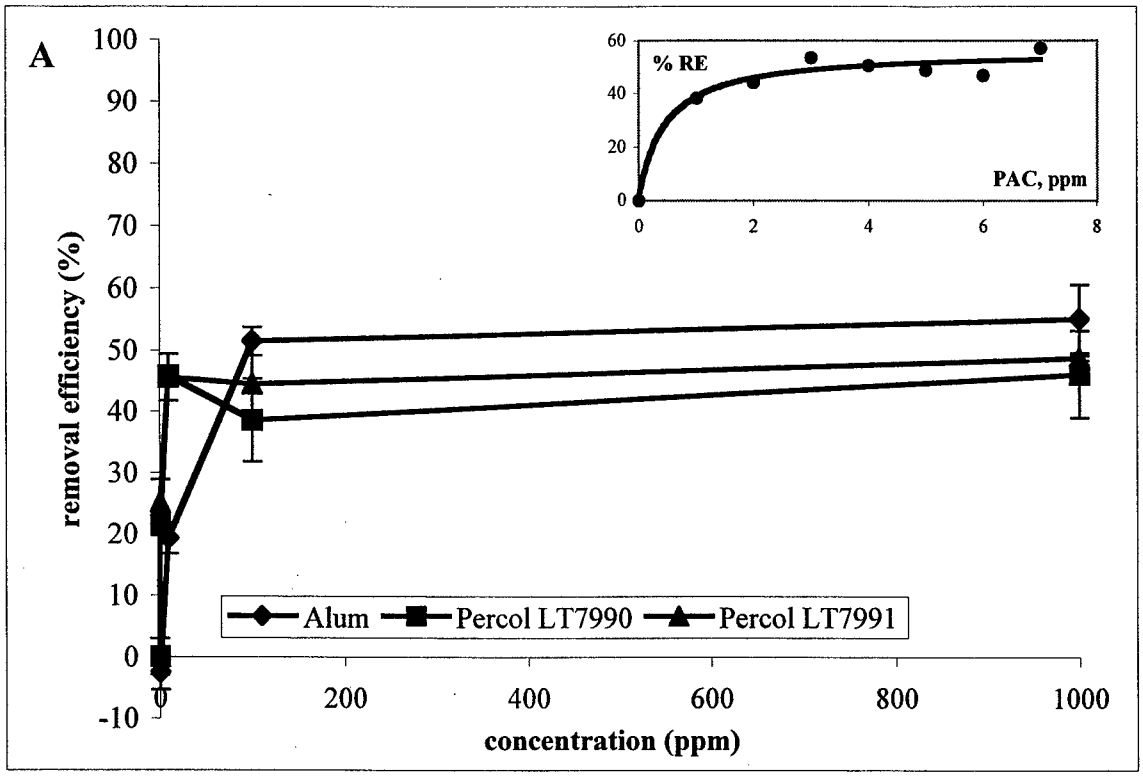

100
80
60
40
20
0
removal efficiency (%)
0.00
0.02
0.04
0.06
0.08
0.10
IMC-P clay concentration (g l-1)
IMC-P only
IMC-P + 5 ppm PAC

Figure 2-3. Removal efficiency (RE) of inorganic coagulants (alum and polyalumnium chloride=PAC) and organic (Percol 720, 778, LT-7990, LT-7991) against *Gymnodinium breve* (A) and *Aureococcus anophagefferens* (B). Inset: PAC removal curve against *G. breve.*


A
removal efficiency (%)
concentration (ppm)
Alum
Percol LT7990
Percol LT7991
% RE
PAC, ppm

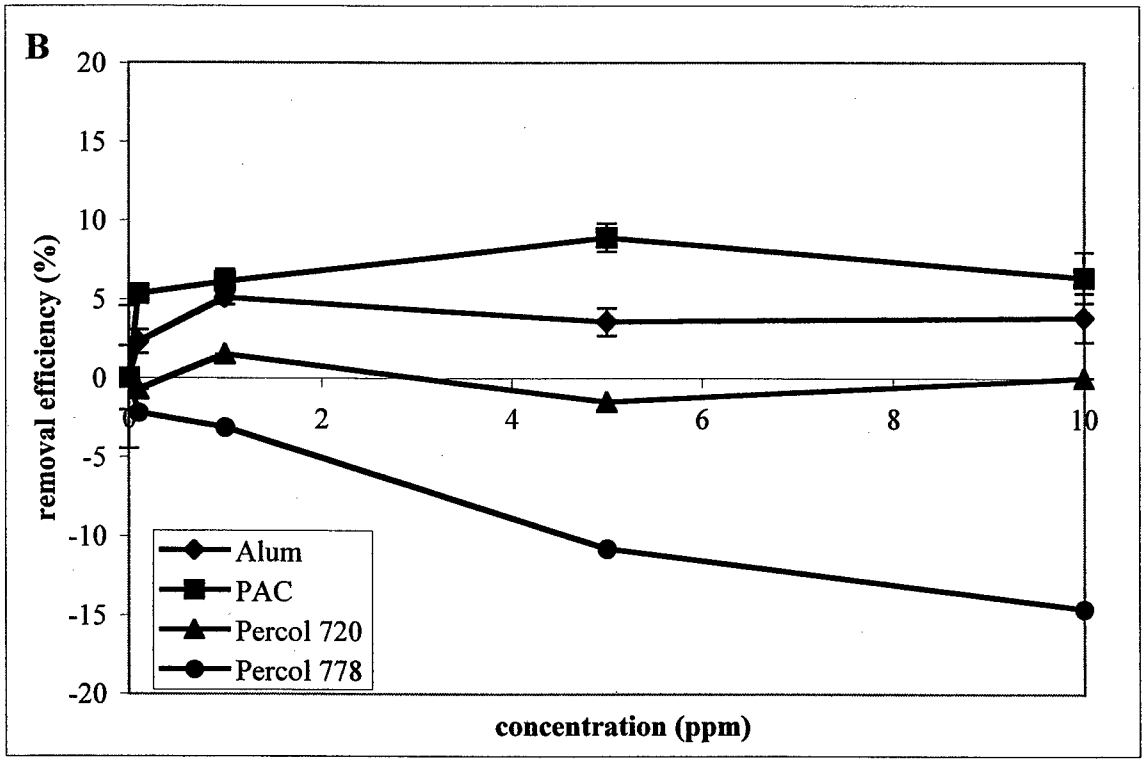

B
removal efficiency (%)
concentration (ppm)
Alum
PAC
Percol 720
Percol 778

Table 2-2. Removal efficiency of clays treated with surfactants against *Gymnodinium breve*. 0.10 g l^{-1} of IMC-P2 phosphatic clay (highly effective) and SW-NZ zeolite (ineffective) were pre-treated with increasing dosage of alum, Percol LT-7990 and Percol LT-7991 prior to addition into cell suspension. For removal efficiency, standard error values (n = 3) are given in parentheses.

Clay	Surfactant	surfactant concentration (ppm)	removal efficiency (%)
IMC-P2 phosphatic clay (0.10 g L^{-1})	alum	clay alone	93.7 (0.85)
		1	93.4 (0.37)
		10	91.8 (2.98)
		100	93.5 (0.74)
		1000	67.6 (2.41)
	Percol LT7990	clay alone	89.2 (2.29)
		1	88.8 (3.08)
		10	50.0 (2.44)
		100	51.2 (2.31)
		1000	64.6 (4.31)
	Percol LT7991	clay alone	85.4 (1.87)
		1	65.4 (3.74)
		10	46.2 (2.44)
		100	42.7 (8.06)
		1000	28.6 (7.29)
SW-NZ Southwestern "Natur" zeolite (0.10 g L^{-1})	alum	clay alone	1.5 (4.35)
		1	5.7 (5.21)
		10	-4.9 (5.21)
		100	-1.7 (4.52)
		1000	3.5 (7.53)
	Percol LT7990	clay alone	1.5 (4.35)
		1	-2.9 (8.81)
		10	0.5 (0.00)
		100	28.1 (7.98)
		1000	33.4 (6.76)
	Percol LT7991	clay alone	1.5 (4.35)
		1	-7.4 (7.73)
		10	12.4 (4.13)
		100	39.0 (12.43)
		1000	35.6 (2.12)

In the study, the zeolite SW-NZ displayed one of the lowest removal rates against *Gymnodinium breve* (20% at 2.0 g l^{-1}). The addition of alum to this 'ineffective' clay increased its removal efficiency relative to clay alone (Table 2-2) but decreased to negative values between 10 and 100 ppm, suggesting the stabilization effect observed in the prior experiment (i.e. Percol 778 alone against *Aureococcus anophagefferens*). The combination of 0.50 g l^{-1} of SW-NZ and 100 ppm of either Percol LT-7990 or LT-7991 greatly improved removal efficiency compared to the clay alone (from 30 to 49%; data not shown). However, this improvement is equivalent to the removal efficiency of either polymer alone.

In this study, there was no combination of clay and coagulant/flocculant that showed significant improvement in the removal of *Aureococcus anophagefferens* (Appendix A-3).

Pulsed clay addition and mixing. Sequential pulsed addition of clays did not significantly improve the removal of *Aureococcus anophagefferens* using H-DP kaolinite (Fig. 2-4). However, the single clay pulse followed by agitation immediately after addition produced higher removal efficiency. This variation in the protocol was the only improvement observed in the removal of *A. anophagefferens* with clays throughout this study. Normally, the clay suspension is added gently to the top of the tube, where it forms a layer that gradually sediments out.

Viability and growth of *Gymnodinium breve* after clay treatment. The number of living versus dead (dying) *Gymnodinium breve* cells based on vital staining is summarized in Fig. 2-5. Immediately after phosphatic (IMC-P2) clay addition (2.5 h), there were no dead cells detected in any of the treatments. Mortality increased dramatically after 12 h, especially at 0.10 and 0.50 g l^{-1}. The number of dead cells was more difficult to count at higher clay loadings because most of the cells were lysed and became indistinguishable from the clay particles in the surrounding medium. Therefore, the mortality values at 0.50 g l^{-1} are likely to be underestimates of cell death. By 24 and 48 h, most of the cells were dead and lysed in the 0.50 g l^{-1} treatment (Fig. 2-5). Cell death was minimal in the control and 0.03 g l^{-1} treatment.

The recovery of *Gymnodinium breve* after treatment was inversely related to the clay dosage (Fig. 2-6). There was no difference between the growth of the controls

(distilled water added to f/2) and 0.03 g l^{-1} clay addition, although 67% of the cells had been sedimented by the clay compared to 0.03% for the controls (data not shown). Despite the similarity in ultimate cell yield, the culture treated with clay showed a 24 h delay in the recovery. This delay increased to 48 h when the clay concentration was doubled to 0.06 g l^{-1}. Recovery and growth ceased when the clay dosage exceeded 0.13 g l^{-1} in the unmixed cultures.

In the resuspension experiments, the fluorescence values were plotted against time for each experiment, and the best-fit regression lines through the points were calculated (Appendix A-1). The slope of these lines was then compared statistically using a Student's t-test. No difference was found in the slope values among the 3 mixing schedules within each clay treatment, except for 0.10 g l^{-1} (Table 2-3). At this dosage, there was a significant difference between daily mixing and every 3 d mixing, and between 2 and 3 d mixing. Following the comparisons of mixing frequency within each clay treatment, the data showing no statistical differences were grouped together and a new regression line was determined. The slope values were then compared among the different clay loadings (Table 2-3). There was no difference between the control and the lowest loading (0.01 g l^{-1}). At 0.20 g l^{-1}, the large slope value was observed which seemed to indicate that a large number of cells escaped from the floc and recovered. The lowest slope value was found at 0.50 g l^{-1} suggesting low cell recovery. At the intermediate amount of clay (0.10 g l^{-1}), the recovery seems to be determined by the frequency of mixing: cell recovery was greatest when the pellet was resuspend daily or within 2 d after treatment. Survival of the cells decreased when the pellet was mixed after the third day.

Causes of mortality. The growth of *Gymnodinium breve* in the clay-treated (filtered) medium was no different from that of controls over the entire range of clay loadings (Fig. 2-7). Clearly, mortality was not due to the release of toxicants.

Removal of other HAB species. IMC-P2 phosphatic clay displayed varying removal ability for the different HAB species tested (Fig. 2-8). As with *Gymnodinium breve*, *Heterosigma akashiwo* was removed with high efficiency. Moderate removal was found with *Alexandrium tamarense*.

Figure 2-4. Effect of agitation and pulsed clay addition on the removal efficiency of clays against *Aureococcus anophagefferens*. Clay pulses were added at 15-min intervals.

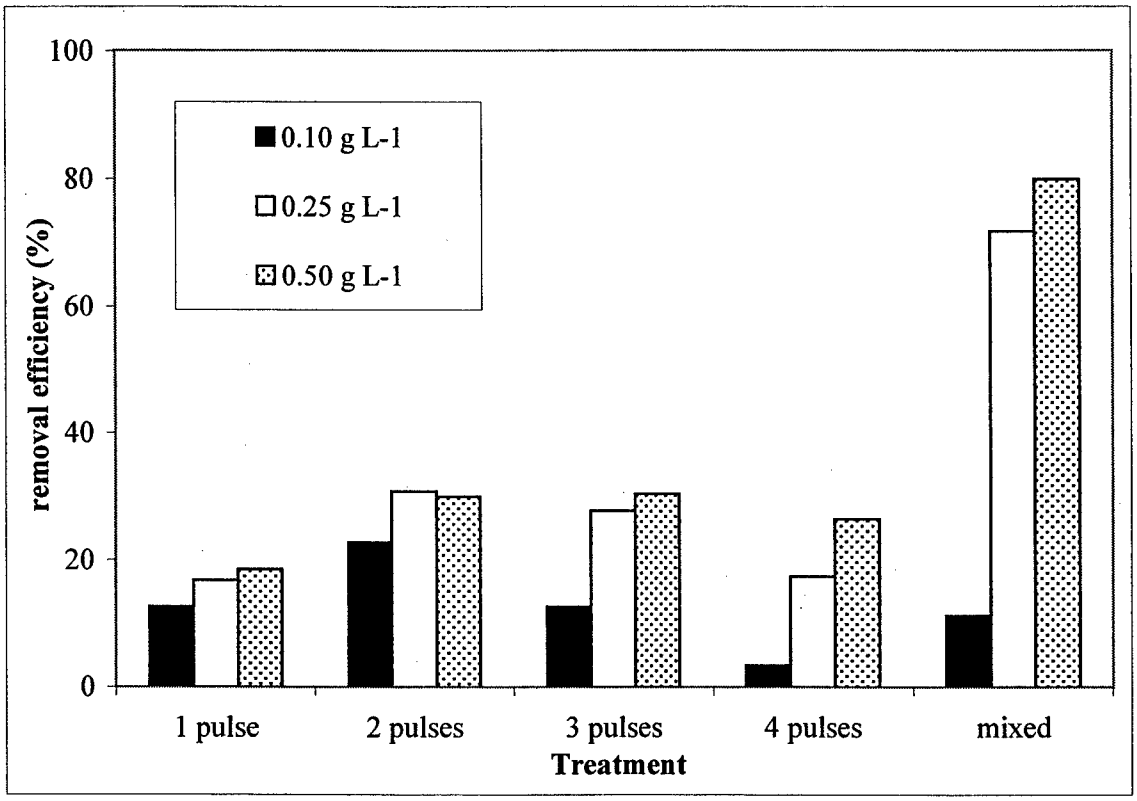

100
80
60
40
20
0
removal efficiency (%)
0.10 g L-1
0.25 g L-1
0.50 g L-1
1 pulse
2 pulses
3 pulses
4 pulses
mixed
Treatment

Figure 2-5. *Gymnodinium breve*. Effect of clay treatment on viability. y-axis indicates the percent dead cells and the removal efficiency (RE) at 2.5 h. Cell viability was determined using 5-chloromethylfluorescein diacetate staining at various intervals after clay addition (2.5, 12, 24 and 48 h).

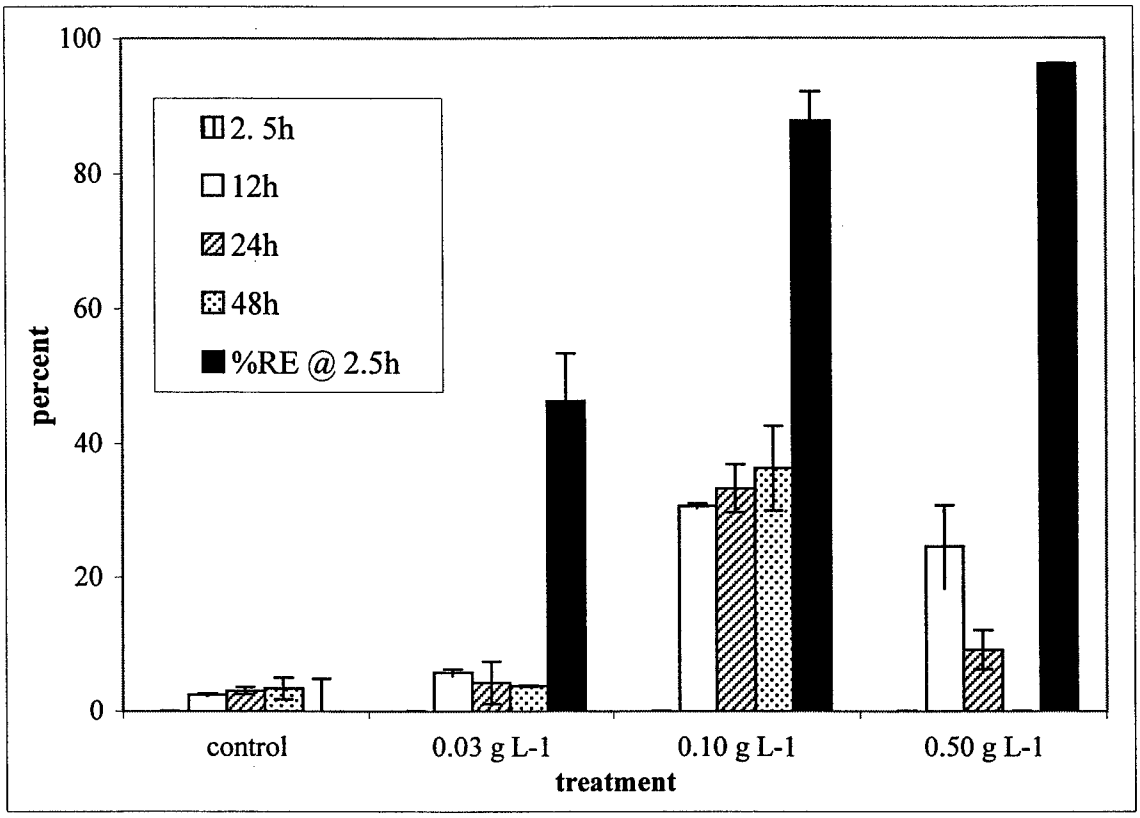

100
80
60
40
20
0
percent
2. 5h
12h
24h
48h
%RE @ 2.5h
control
0.03 g L-1
0.10 g L-1
0.50 g L-1
treatment

Figure 2-6. *Gymnodinium breve*. Long-term growth following clay treatment. RFU: relative fluorescence units.

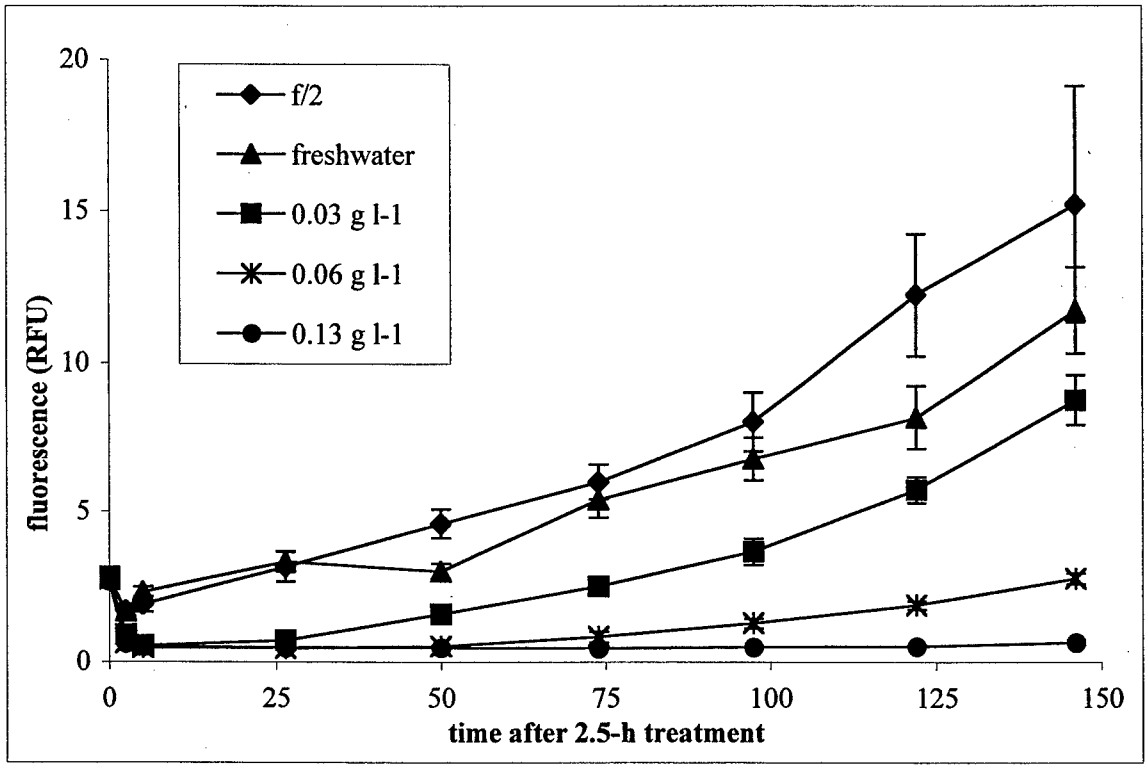

20
15
10
5
0
fluorescence (RFU)
f/2
freshwater
0.03 g l-1
0.06 g l-1
0.13 g l-1
0
25
50
75
100
125
150
time after 2.5-h treatment

Table 2-3. *Gymnodinium breve*. Resuspension experiment to determined cell recovery after treatment with IMC-P2 phosphatic clay. Results were analyzed statistically using linear regression and Student's t-test (Sigmastat). Within each clay treatment, there was no significant difference among the 3 mixing schedules (daily, every 2 d and every 3 d) except at 0.10 g l^{-1} loading. Subsequently, all data for each treatment were combined (n = 21), except for 0.10 g l^{-1} data set, in which they were considered separately (n = 7). Data below represent the mean slope (change in cell concentration/time) of the linear regression for each treatment, standard error and the sample size (n). Cell concentration was determined by fluorescence. Slopes were compared by a pairwise Student's t-test. Treatments with the same letter did not differ significantly from each other ($p > 0.05$), treatments with different letters differed significantly.

Treatment ($g\ L^{-1}$)	mean slope (change in cell density t^{-1})	n	S.E.
0.00	43.33 (A)	21	2.14
0.01	47.48 (A)	21	4.50
0.10 (daily)	127.74 (B)	7	6.91
0.10 (every 2 days)	124.12 (B)	7	5.54
0.10 (every 3 days)	90.57 (C)	7	2.55
0.20	248.7 (D)	21	14.54
0.50	9.72 (E)	21	1.45

Figure 2-7. *Gymnodinium breve*. Cell growth in conditioned seawater following *G. breve*/clay experiment. One control corresponded to clay-treated seawater (0.10 g l^{-1}) without cells, while second control contained cells treated with 1 ml of distilled/deionized water without clay.

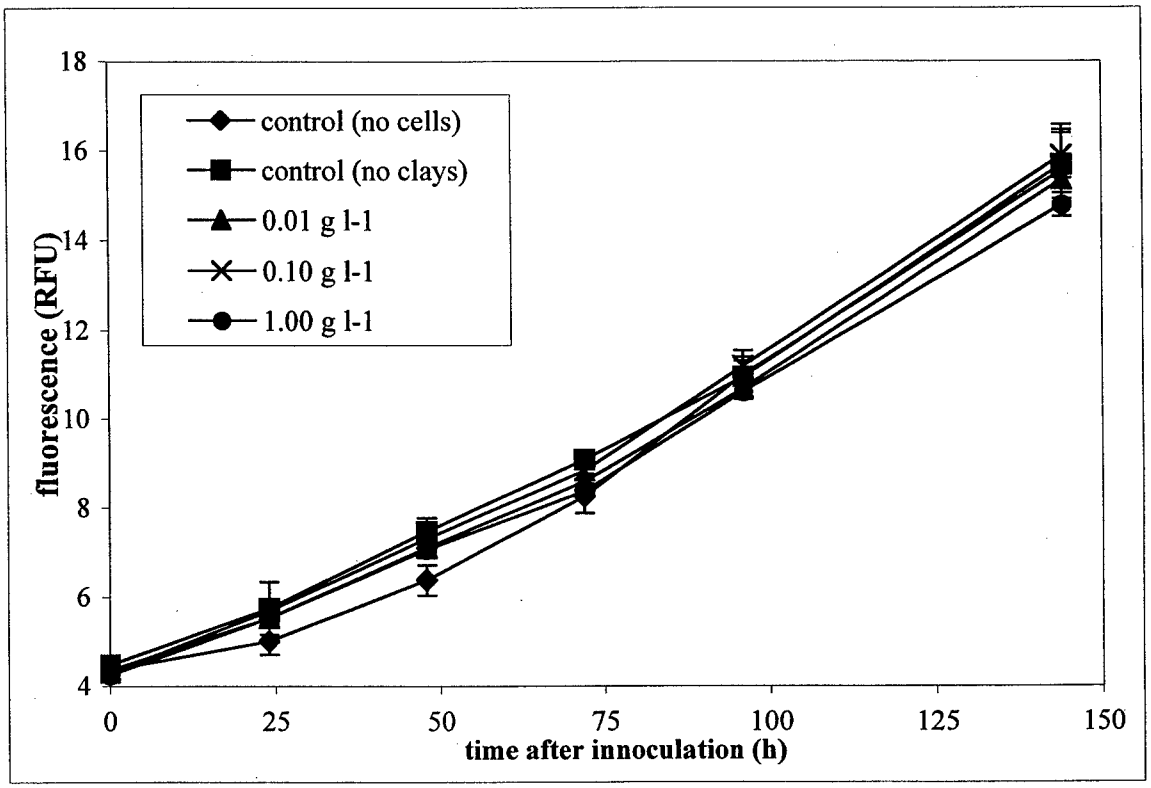
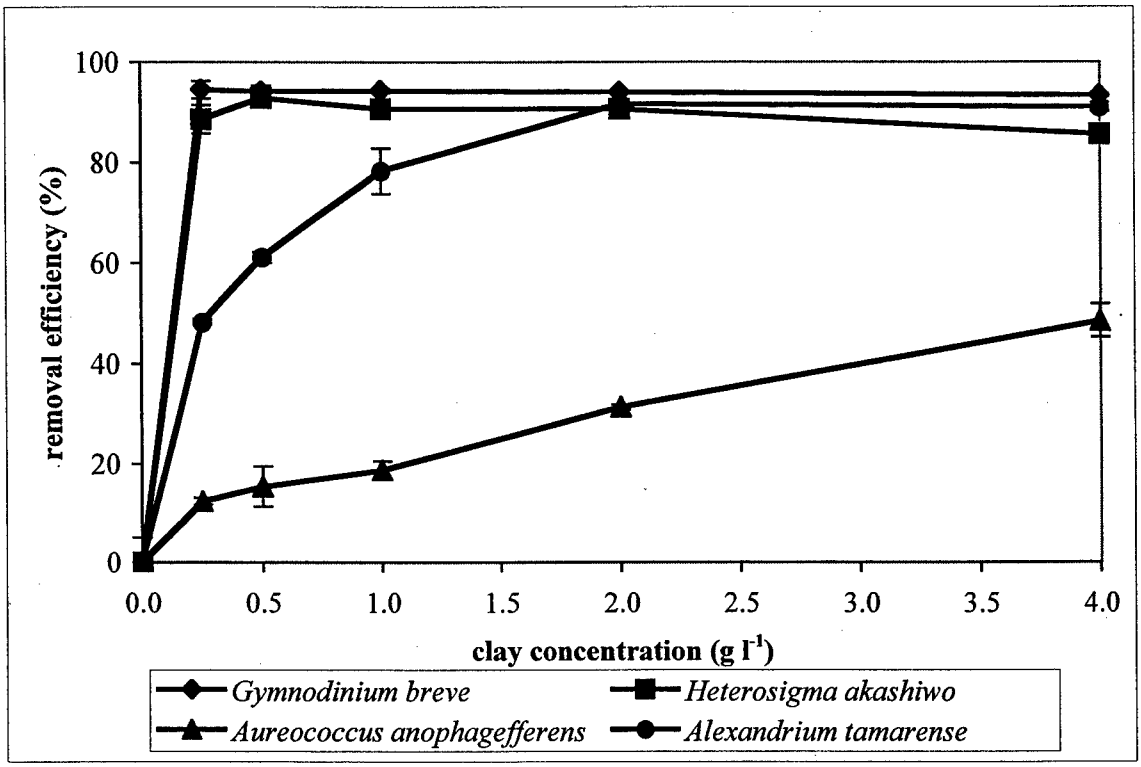

control (no cells)
control (no clays)
0.01 g l-1
0.10 g l-1
1.00 g l-1
fluorescence (RFU)
time after innoculation (h)
18
16
14
12
10
8
6
4
0
25
50
75
100
125
150

Figure 2-8. Removal efficiency of IMC-P2 (Florida phosphatic clay) against several HAB species.


100
80
60
40
20
0
removal efficiency (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
clay concentration (g l-1)
Gymnodinium breve
Heterosigma akashiwo
Aureococcus anophagefferens
Alexandrium tamarense

Discussion

Clay screening. This study demonstrated that different clay minerals have different removal ability for the range of HAB species tested. In addition, a clay mineral that exhibits high removal efficiency against one organism may not behave similarly against another organism. Therefore, the most suitable clay(s) for a given target species must be determined empirically.

In the case of *Gymnodinium breve*, the most effective clays were the bentonites, montmorillonites, and the Florida phosphatic clays which mostly contain montmorillonite (Bromwell, 1982). Their removal curves followed a hyperbolic function that quickly plateaued near 90% efficiency after exceeding 0.25 g l^{-1} loading (Fig. 2-1). Structurally, these clays are composed of 3 sheet-like layers which have a high swelling index (i.e. the ability to absorb and retain water between the layers, causing an expansion in the crystal). This property may increase the contact frequency between the clay particles and the algal cells, since larger particles can sweep through a larger surface than smaller particles.

By comparison, the zeolites and kaolinites have low swelling indices which may explain why these clays have much lower removal efficiencies against *Gymnodinium breve* (Table 2-1). However, 1 zeolite (SW-NM) and 1 kaolinite (H-DP) displayed removal ability comparable to the clays in Group 1. SW-NM appeared as a very fine powder compared to the other zeolites, which seemed coarser and contained sand-sized grains. SW-NM remained in suspension longer, while the others sank rapidly, allowing the former to have more possible interactions with the algae. On the other hand, the effectiveness of H-DP may be explained by surface chemistry and a potentially higher affinity for the cells. H-DP is the only clay tested which was treated with strong acids during its production. In fact, the clay suspension prepared with distilled/DI water (pH = 6.98) was acidic (pH = 4.86), indicating that the clays may carry residual protons which were later released into the medium. This charge on the clay surface may increase its 'stickiness' or surface reactivity with the algal cells.

Moreover, H-DP was the clay which displayed one of the best removal efficiencies (at 0.25 g l^{-1}) against *Aureococcus anophagefferens*, although the values did not exceed 40%. Loess clay (LO, kaolinite) and MI-HY (attapulgite) produced similar results (Fig. 2-1B). In this study, 85% was the highest removal efficiency observed

was achieved with 4.0 g l^{-1} of Loess clay, 16 times more clay than in *Gymnodinium breve*. As with *G. breve*, the zeolites were ineffective against *A. anophagefferens* (Appendix A-2). Aside from this observation, however, there was no clear pattern between removal effectiveness and mineral type (montmorillonite/bentonite vs kaolinite; Table 2-1).

This resistance of *Aureococcus anophagefferens* to clay flocculation may be explained by 2 physico-chemical factors: (1) lower contact efficiency due to low 'stickiness' of the organism, and (2) low contact frequency between clays and cells due to the small cell size (ca. 2 μm). Direct measurements of algal 'stickiness' are difficult to make, and there have been no determinations for *A. anophagefferens*. Therefore, this conjecture must be addressed in a future report. In the second hypothesis, the small size of the organism is a critical factor. The contact frequency between the particles is governed by 3 factors (O'Melia and Tiller, 1993): Brownian diffusion, water motion (laminar and turbulent), and differential sedimentation. In this system, Brownian diffusion can be disregarded since it is insignificant when particles exceed 1 μm. Likewise, the effect of water motion is minimal since flocculation proceeded under quiescent conditions. Thus, the number of clay-cell contacts will be dominated by differential sedimentation (collisions produced when smaller particles are intercepted by larger, more rapidly-sinking particles). For this to occur, a difference in size between particles is necessary to create particles with different sinking rates. However, as the size of one particle greatly exceeds that of the other, the larger particle displaces more water (hydrodynamic effect), making it more difficult for the smaller particle to approach, interact and bind with the larger one (Thomas et al., 1999). With this reasoning, we hypothesized that clay-clay flocculation occurred at the water surface after it was added to the culture. As the clay flocs sank through the water column, they interacted with the cells and removed them. As the floc size increased along the tube and greatly surpassed the cells' size, the contact frequency between the cells and clays diminished, leaving most of the cells behind. This is consistent with our observation that by gentle mixing of the cell/clay suspension just after clay addition - a process that kept initial clay size small and increased the collision rates (by turbulent water motion) - significantly more *A.*

anophagefferens was removed from the medium (Fig. 2-4). Therefore, we favor this explanation at this juncture.

Coagulants/flocculants without clays. While coagulants and flocculants are highly effective in water-treatment facilities in flocculating and quickening the removal of fine suspended particles, the substances tested in this study were ineffective against *Gymnodinium breve* (51% removal efficiency) and *Aureococcus anophagefferens* (10% removal efficiency) (Fig. 2-3). In theory, coagulants and flocculants promote flocculation by affecting the surface chemistry (stickiness) of the particles. Coagulants reduce electrostatic repulsion by collapsing the electrical double layer surrounding the charged particle, allowing attractive forces and aggregation to dominate. Flocculants can induce flocculation by acting as interparticle bridges between 2 or more particles which may otherwise repel one another. Indeed, the addition of these substances may have increased the propensity towards flocculation during the experiment. However, increasing the flocculation rate would also require an increase in interparticle contacts (e.g. through mixing or agitation), which was not altered in these experiments. Moreover, the addition of clays would have increased the total number of particles in the system (in the order of 10^{13} to 10^{15} 1 μm-sized particles), and hence, the chance of collisions producing larger particles. Without interparticle contacts to create progressively larger particles, the addition of coagulants and flocculants produced no apparent effects on the system.

Other explanations include spatial separation between the substances added at the surface (unmixed), and the target cells distributed throughout the medium. Alternatively, the high ionic strength and alkalinity of the seawater may have rendered the chemicals ineffective or inert.

In the case of *Aureococcus anophagefferens* treated with flocculants (Percol 720 and 778), negative values of removal efficiency were found (Fig. 2-3B). Based on the method of calculating removal (Eq. 2-1), we concluded that the cells treated with flocculant sank less than those in the control (no flocculant). This result suggested that the system became more stable, and sinking was retarded through an increase in seawater viscosity originating from the addition of flocculant itself. This effect was apparent after

the experiment when cultures were discarded and the seawater in the tubes appeared thicker and more difficult to pour out.

Coagulants/flocculants with clay. The most effective clays and 1 ineffective clay were treated with varying amounts of coagulants and flocculants prior to being added to the cell suspension. This combination was predicted to enhance the clays' effectiveness by increasing their chemical affinity (or stickiness) for the HAB cells. In the case of *Gymnodinium breve* and IMC-P2, a highly effective clay, the addition of polyaluminum chloride (PAC) at 5 ppm enhanced removal efficiency by 1 order of magnitude (Fig. 2-2). In contrast, alum, Percol LT-7990 and Percol LT-7991 reduced removal efficiency. The primary effects of adding these chemicals above 10 ppm were the rapid flocculation of the clay slurry prior to addition, the apparent increase in clay-clay flocculation, and the rapid sedimentation of the flocs, thereby limiting their residence time in the water column and the potential contacts with cells.

The addition of coagulants/flocculants slightly enhanced the removal of SW-NZ zeolite against *Gymnodinium breve* (from 20 to 50%). However, the higher values only matched those attained when flocculant was added alone (Table 2-2). Nevertheless, this improvement demonstrates the potential usefulness of coagulants and flocculants, especially in the case of PAC and IMC-P2. Interestingly, PAC alone was not effective (Fig. 2-3A: inset). The explanations for this observation may be similar to those offered in the previous section, where coagulants and flocculants were used alone. In this example, the addition of PAC enhances the chemical interaction of the surface, but the clays themselves act as the 'ballast' which eventually promotes the sinking of the cell.

The various combinations of clay and coagulants/flocculants against *Aureococcus anophagefferens* were all ineffective (Appendix A-3). Likewise, the gradual and sequential addition of clays did not produce a significant increase. The only substantial improve-ment in removal efficiency occurred when H-DP was added to the culture and dispersed thoroughly in the medium by gently mixing (Fig. 2-4). Presumably this step increased interparticle collisions and prevented the flocs from growing too fast.

Viability and growth of *Gymnodinium breve* after clay treatment. Based on our studies, the mortality of *Gymnodinium breve* after treatment with IMC-P2 depended on 3 factors. In decreasing order of importance, survival was influenced by the clay loading,

the duration of contact between cells and clays, and the opportunity of escape through resuspension. At relatively low clay loadings (≤ 0.03 g l^{-1}), cell mortality was small (Fig. 2-5), and the organisms were able to gradually, but completely, free themselves from the settled clay matrix, swim away, and resume vegetative growth. In Fig. 2-6, their escape is documented as a rapid increase in cell concentration within 24 h, which cannot be explained by simple growth and division. Moreover, this escape can occur with or without the aid of manual resuspension.

At relatively high clay loadings (≥ 0.50 g l^{-1}), cell mortality rapidly increased after 2.5 h of exposure to the clay (Fig. 2-5). Despite the large number of cells contained within the clay matrix, few cells escaped. After 24 h without resuspension, most of the cells died (Fig. 2-5). This is also evident from the very low recovery in the mixing experiment even with daily resuspension (Table 2-3).

The interplay of clay dosage, duration of contact and resuspension is more complicated at intermediate clay loadings (0.05 to 0.25 g l^{-1}). Cell death markedly increases with increasing dosage, with significant mortality after 2.5 h, especially if resuspension does not occur in the first 24 h (Fig. 2-5, Table 2-3). At 0.06 g l^{-1}, the cells survived and escaped the clay matrix without resuspension, although their recovery was delayed by 48 h (twice the amount of time for double the clay loading (Fig. 2-6). At 0.13 g l^{-1} with no resuspension, no recovery was seen even after 6 d (Fig. 2-6). However, if resuspension took place within 3 d after clay addition, the cells survived and recovered, although their subsequent growth was better if resuspension happened sooner (Table 2-3). Finally, at 0.20 g l^{-1} (Table 2-3), escape and recovery did not take place without resuspension (Fig. 2-6, same as 0.13 g l^{-1}), but the cells survived in the matrix and grew even after waiting 3 d before resuspending the pellet.

A preliminary investigation on the possible cause of cell mortality did not indicate the release of cytotoxic substances from the clay into the surrounding medium. Moreover, fresh *Gymnodinium breve* cultures innoculated into conditioned water (i.e. water filtered following clay treatment) did not show growth inhibition or cell loss (Fig. 2-7). Cell death and lysis was evident within the settled clay matrix from vital staining and direct microscopic observation. The cells appeared moribund, and stained cytoplasmic material could be seen leaking into the medium. We conclude that cell death

was caused by the direct physical contact between the cells and clays at very high dosage and over extended periods of time. However, the exact means by which surface interactions can lead to cell death remains unknown. In earlier studies, cell lysis by clays may have resulted from the presence of prosthetic groups, such as aluminum, on the clay surface. Shirota (1989b) argued that aluminum released from clays can cause fragile cells to lyse. Alternatively, acid/base reactions on the clay surface can lead to rapid changes in pH which may cause serious damage to the integrity and function of the cell membrane.

Phosphatic clays. Despite differences in their geographic origins and methods of processing, the 5 samples of Florida phosphatic clays exhibited consistently high, statistically similar removal efficiencies of *Gymnodinium breve* (Table 2-1). They are a by-product of phosphate mining, a major industry across central and northern Florida. The 'clay' itself is a freshwater suspension, composed of silt and sand (30 to 50%), and a so-called clay-sized fraction (<2 μm) making up to two-thirds of the solid mass (Bromwell, 1982). The major minerals in the clay fraction are smectite (i.e. montmorillonite, 8 to 32%), illite (0 to14%), palygorskite (0 to 23%) and kaolinite (0.8 to 1.9%), with some minor minerals such as wavellite, crandellite, dolomite/calcite, feldspar, millisite, iron phosphates and trace amounts of various metals (Barwood, 1982). Generally, the material leaves the beneficiation plant as a slurry of 3% solids, which is then stored in ponds for settling and dewatering, producing clay with up to 55% solids in older basins. This clay material is the most promising choice against the Florida red-tide organism, not only because of its effectiveness, but also because of its availability in the affected region and the large available supply of the material.

Presently, there is no clear explanation to account for the effectiveness of this clay. It is similar to the other clays in Group 1 in that montmorillonite is its main constituent mineral. One reason may lie in its chemical composition which may include organic matter that can enhance its 'stickiness'. Microscopic videography of the clay-cell flocculation *in situ* showed that the organisms seemed to adhere quickly and strongly to the clay flocs upon contact, becoming trapped and reducing the chance of escape during their rapid descent. These issues are currently being investigated.

Lastly, the phosphatic clays appeared to have varying affinity towards different HAB species (Fig. 2-8). *Gymnodinium breve* and *Heterosigma akashiwo* were highly affected, while *Alexandrium tamarense* was only moderately removed. The clay seemed ineffective against *Aureococcus anophagefferens*. This characteristic may have practical implications in clay use: it demonstrates the versatility of the clay to target specific HAB organisms and not to remove all algal species indiscriminately.

Potential for clays in HAB management. This study demonstrated that clays can effectively remove a number of HAB species that threaten US coasts. For example, the Florida phosphatic clays are very effective against *Gymnodinium breve* because of their consistent effectiveness at low loading. Clay loading may be further reduced by adding minute amounts of PAC, a chemical additive approved for drinking water treatment (ANSI/NSF Standard 60). Clay treatment can also kill cells at the relevant dosages and could minimize the chance of bloom recurrence. Moreover, these clays displayed varying removal ability against different algal species, suggesting the possibility of selective removal. Logistically, clay is a suitable mitigation candidate because it is plentiful, inexpensive, and readily available close to the area where it may be used in Florida. Phosphatic clay also showed promise against *Heterosigma akashiwo*, although more tests are needed.

Assuming that removal efficiency for phosphatic clays remains constant with increasing dimensions and scale of the water column, a target clay loading of 0.05 g l^{-1} (without coagulant) would remove 85% of *Gymnodinium breve* cells. For a basin with a surface area of 1 km^2 cleared to a depth of 4 m (total volume = 4.0×10^6 m^2 or 4×10^9 l), approx. 200 metric tons of clay would be required for 1 treatment. If all this material fell straight to the bottom and deposited evenly over the given surface area, the loading would be 200 g m^2. Realistically, winds and currents would undoubtedly spread this loading over a much larger area. Finally, the addition of polyaluminum chloride to phosphatic clay can reduce the target loading to 0.01 g l^{-1} (75% removal efficiency), giving a mass of 40 metric tons of clay, which would settle and cover the bottom at 40 g m^2.

The case is not so clear for *Aureococcus anophagefferens*. While higher removal efficiencies were finally attained for this organism (i.e. 80% with H-DP kaolinite),

mixing and thorough dispersal of the clay into the medium were necessary. This step required energy and may create logistical and practical complications if the clay is to be used in the field. Future progress in the use of clays against this species may rely on a better understanding of the mechanism of removal and the factors that influence the clay-cell flocculation, some of which may have been identified during this study (i.e. contact rate, initial size of the clay particle, surface charge effects).

Finally, we recognize the need to investigate the possible impacts of clay addition to the marine environment and ecosystem, especially in the benthos. New studies are currently underway that address these issues. These and other projects will be critical in providing the scientific data needed to evaluate the possible use of clays in mitigating the impacts of HABs.

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CHAPTER 3

The Differential Removal of Marine Algal Species by Clay Aggregation: Effects of Algal Concentration, Size and Swimming Rate

Abstract

Clay minerals have the ability to physically remove bloom-forming algae from the water column through mutual aggregation and sedimentation. In this study, 17 species representing five taxo-nomic classes and a range of morphological and behavioral characteristics were treated with Florida phosphatic clay (IMC-P2) to determine their removal efficiency (RE). For all species, the RE increased with clay loading, and for most species, increased with cell concentration. Among the flagellate species, *Karenia brevis* (= *Gymnodinium breve*) and *Akashiwo sanguinea* (= *Gymnodinium sanguineum*) showed the highest RE (> 77%) when clay loading exceeded 0.10 g L^{-1} and the cell concentration was 1000 cell ml^{-1}. RE was poorly correlated with cell length ($R^2 = 0.15$), the projected cross-sectional area ($R^2 = 0.23$) and swimming speed ($R^2 = 0.04$). For flagellate species, however, the RE was correlated with the total collision frequency coefficient ($R^2 = 0.90$), calculated from aggregation theory, which incorporates both size and swimming speed. There were no other distinguishable patterns between a clay's effectiveness and the type of outer structures of the cell (i.e. theca, silica frustule, cell wall, cell membrane). These results demonstrated that this montmorillonite-rich phosphatic clay did not remove phytoplankton species equally well and that its effectiveness for a given species was not correlated to the organism's linear dimensions or its swimming speed. Nevertheless, the motility of the organism may be an important mechanism for increasing the collision rate between the clay particles and the cells, particularly during the initial stages of aggregation when clay particle sizes are relatively small (<50 μm). However, the role of cell stickiness and clay surface chemistry were not addressed in this study and could not be discounted.

In mixed culture experiments, *Karenia brevis* was combined with either the dino-flagellate, *Prorocentrum micans*, or the diatom, *Skeletonema costatum*. IMC-P2 phospha-tic clay preferentially removed *K. brevis* (> 80% RE) even as the concentration of the other species was increased. By comparison, the removal of *P. micans* and *S. costatum* remained moderate (< 50% RE). In addition, the presence of the other species increased the removal of the *K. brevis* relative to *K. brevis* alone. In mesocosm studies, the remov-al efficiency of *K. brevis* (49% at 0.05 g L^{-1} of clay) was higher than predicted by labora-tory studies given the low cell concentrations (< 200 cell ml^{-1}). The two dominant

diatoms, *Skeletonema* sp. and *Bacillaria* sp., were also removed by clay, but not at high efficiency (55.2% and 34.1%, respectively). However, a small species such as *Prymnesium* sp. was relatively unaffected, although the high variability in the data made this observation tenuous. These results demonstrated differential removal of algal species in a mixed assemblage both in laboratory and mesocosm experiments and identified the underlying mechanism to be a combination of size, swimming speed and cell concentration.

Introduction

Harmful algal blooms (HABs) are natural aquatic phenomena caused by the rapid proliferation and accumulation of certain species of microalgae, many of which have deleterious effects on public health, industry (e.g. aquaculture, shellfish fisheries, tourism), and the quality of freshwater and marine environments. In recent years, there have been successful efforts in using clay minerals to physically remove HABs from suspension as a way of mitigating their impacts (Maruyama et al, 1987; Yu et al., 1994a; Na et al., 1996; Sengco et al., 2001). The principle is based on the mutual aggregation between the algal cells and the mineral particles, leading to the formation of large flocs that rapidly settle to the ocean floor (Degens and Ittekot, 1984; Shirota, 1989). Clay minerals were selected for this purpose because they are natural substances which were considered a low risk for environmental damage (Portman, 1970; Howell and Shelton, 1970; McIntyre, 1983). Moreover, they are abundant, easy to handle, relatively inexpensive, and available in large quantities. Clays have been used effectively in the field to protect the mariculture industry in the Seto Inland Sea of Japan (Shirota, 1989) and in South Korea (Bae et al., 1998; Choi et al., 1998) from a number of fish-killing HAB outbreaks. Clay minerals such as montmorillonite and clay mineral-bearing yellow loess (i.e.a mixture of gibbsite, quartz and kaolinite) eliminated over 90% of the bloom density to a depth of 4 m with no reported mortality in the caged fish due to clay treatment (Shirota, 1989; Na et al., 1996). Within hours, the transparency of the water column improved, followed by the recovery of the moribund fish. Based on these reports and current research programs, clay dispersal has emerged as one of the most promising strategies for controlling HABs directly by treating the causative organisms (Anderson, 1997).

From empirical studies, pure montmorillonites and deposits containing a large proportion of montmorillonite (e.g. Florida phosphatic clay) consistently exhibited the greatest ability to remove a variety of algal species with moderate to high efficiency (Avnimelech et al., 1982; Maruyama et al., 1987; Na et al., 1996; Yu et al., 1994b; Sengco et al., 2001). Yu et al. (1994b) reported that the removal of two diatom species, *Nitzschia pungens* (79%) and *Skeletonema costatum* (62%) with montmorillonite (at 0.25 g L^{-1}) was greater than those of two dinoflagellates, *Prorocentrum minimum* (25%) and

Noctiluca scintillans (15%). They proposed that the larger size (via chain formation) and presence of elaborate projections in the diatoms contributed to a higher "specific" surface area to which the mineral particles could attach. Recently, Sengco et al. (2001) reported different removal efficiencies for *Heterosigma akashiwo*, *Gymnodinium breve* (now *Karenia brevis*) and *Alexandrium tamarensis* treated with Florida phosphatic clay. Both *H. akashiwo* and *K. brevis* showed >90% removal efficiency at low clay loading (0.25 g L^{-1}) while the larger *A. tamarensis* only displayed 48%. In another study, the cyano-bacterium *Anabaena* sp. and chlorophyte *Chlorella* sp. showed removal > 90% at 0.20 g L^{-1} of sodium bentonite despite their small sizes (Avnimelech et al., 1982). Given these differences, the possible direct relationship between algal size and removal efficiency with clays remains tentative. Moreover, direct comparisons among the various studies may be difficult due to differences and/or unspecified experimental conditions such as cell concentration and the initial clay particle size.

Despite the growing number of empirical studies, there have only been a few attempts to systematically apply the basic concepts of aggregation theory to the clay-algae system in order to understand the removal process (e.g. Yu et al., 1994a, 1995b). In most cases, the aggregation phenomenon has been described qualitatively, noting the relative importance of particle size, surface properties, concentration, and the chemistry of the aqueous medium. Hydrodynamics were often ignored. Earlier work focused on the aggregation of clay (colloidal) suspensions (Hahn and Stumm, 1970; Thomas et al., 1999), which were later expanded to describe the dynamics of algal-bloom aggregation (Jackson, 1990; Jackson and Lochmann, 1993). However, the mutual aggregation of mineral and organism have not received rigorous, quantitative treatment.

Generally, aggregation is divided into two sequential steps, namely transport and attachment (O'Melia and Tiller, 1993). The main transport mechanisms are Brownian diffusion, shearing and differential sedimentation (Table 3-1). The effectiveness of each mechanism has been linked to particle size (McCave, 1984): Brownian diffusion (perikinetic aggregation) dominates when particle sizes are less than 1 μm. Differential sedimentation is more important at larger particle sizes. Both velocity gradients (orthokinetic aggregation) and differential sedimentation are in effect at intermediate sizes and dominance will be determined by the shear rate (G). Jackson and Lochmann

(1993) also considered collisions due to cell motility. Therefore, this parameter may be important in the collision frequency between flagellated species and clays.

Following transport, attachment occurs between colliding particles depending on their surface properties, and the chemical properties of the medium (e.g. pH, ionic strength). Clay particles develop surface charge from isomorphic substitutions (i.e. exchange of ions with different valences), exchange of ions at the mineral surface and the specific adsorption of charged molecules (e.g. polyelectrolytes, organic matter). The surface charges are balanced by ions of opposite charge in the medium (i.e. counterions) to create the familiar electrical double layer arrangement. In a stable suspension, the interaction between similarly-charged double layers of different particles results in electrostatic repulsion and a low propensity for aggregation. As the concentration of counterions or polyelectrolytes increases, the thickness of the double layer decreases, reducing repulsion, and allowing attractive forces (e.g. van-der-Waals/London forces) to dominate for rapid aggregation. In the high ionic strength of seawater, the thickness of the clay electrical double layer is very small, enabling close approach of particles and effective dominance of attractive van der Waals forces over electrostatic repulsion (Stumm and Morgan, 1996). For algal cells, the surface charge is generated by the presence of various organic molecules such as carbohydrates, glycoproteins and amino acids (Maruyama et al., 1987). Direct measurements of charge have found freshwater species to be electronegative (Tenney et al., 1969). Low aggregation rates in algal suspensions may be achieved by steric stabilization which occurs when organic molecules on the cell surface extend into the medium beyond the double layer (O'Melia and Tiller, 1993). The interaction of these molecules between particles can prevent attachment. In the case of minerals and algal cells, Avnimelech et al. (1984) proposed that organic matter can act as polymer links or bridges between clays and cells to promote attachment.

The first objective of this paper was to compare the individual removal efficiencies (RE) of various algal species with a single phosphatic clay, a montmorillonite-rich product of phosphate extraction in Florida. The null hypothesis was that the removal efficiency (RE) of different marine phytoplankton increases with increasing cell size

Table 3-1. Collision frequency mechanisms for clay-cell aggregation.

A. Brownian diffusion (O'Melia and Tiller, 1993)

$$\beta_{bd}(i,j) = \frac{2kT}{3\mu} \frac{(d_i + d_j)^2}{d_i d_j}$$

where: k = Boltzmann's constant = 1.381 x 10^{-23} kg m^2 s^{-2} K^{-1}
T = absolute temperature in Kelvin = 298 K
μ = dynamic viscosity (29.6 salinity, 25 C)
= 9.4579 x 10^{-4} kg m^{-1} s^{-1}
d_i = cell diameter (across the girdle) in meters
d_j = clay diameter in meters

B. Fluid motion (O'Melia and Tiller, 1993)

$$\beta_{vg}(i,j) = \frac{1}{6} G (d_i + d_j)^3$$

where: G = shear rate in s^{-1}
d_i = cell diameter (across the girdle) in meters
d_j = clay diameter in meters

C. Differential sedimentation (O'Melia and Tiller, 1993)

$$\beta_{ds}(i,j) = \frac{\pi g}{72\mu} (\rho_{clay} - \rho_{water}) (d_i + d_j)^3 |d_i - d_j|$$

where: g = gravitational constant = 9.8 m s-2
μ = dynamic viscosity (29.6 salinity, 25 C)
= 9.4579 x 10^{-4} kg m^{-1} s^{-1}
ρ_{clay} = clay density = 2640 kg m^{-3}
ρ_{water} = seawater denstiy (29.6 salinity, 25 C)
= 1019.3 kg m^{-3}
d_i = cell diameter (across the girdle) in meters
d_j = clay diameter in meters

D. Cell motility (Jackson and Lochmann, 1993)

$$\beta_{sm}(i,j) = 2\pi (D_{c,i} + D_{c,j}) (d_i + d_j)$$

where: d_i = cell diameter (across the girdle) in meters
d_j = clay diameter in meters
$D_{c,i}$ = diffusion coefficient for motile cell

$$D_{c,i} = \frac{1}{3} \nu_c^2 \tau_0$$

where: ν_c = swimming speed in m s^{-1}
τ_0 = length of time for average run = 1 s

$D_{c,j}$ = diffusion coefficient of clays (Elimelech et al., 1995)

$$D_{c,j} = \frac{kT}{3\pi\mu d_j}$$

where: k = 1.381 x 10^{-23} kg m^2 s^{-2} K^{-1}
T = 298 K
μ = 9.4579 x 10^{-4} kg m^{-1} s^{-1}

(i.e. the projected cross-sectional area), the reason being that larger cells are predicted to have higher encounter rates with clay particles than smaller ones, leading to higher co-aggregation rates. In addition, the theoretical collision frequency factor is calculated for a range of clay particle sizes (McCave, 1984) and was correlated to the empirical RE's. The contribution of cell motility to the collision frequency was also considered in the estimates (Jackson and Lochmann, 1993). The second objective of this paper was to examine the RE of two co-occurring species in the same culture relative to the removal when only one is present. The final objective was to examine differential removal of phytoplankton species in a mixed plankton assemblage in mesocosm tanks during a *Karenia brevis* bloom.

Materials and Methods

Algal cultures, size measurements and swimming speed. Cultures were obtained from various sources (Table 3-2) and were grown in batch cultures using modified f/2+Si medium under conditions described by Anderson et al. (1999). Growth was monitored using in vivo cellular fluorescence (Model 10-AU Fluorometer, Turner Designs, Sunnyvale, California, USA) calibrated against microscope cell counts. *Synechococcus* WH8017 was grown in SN-media following the procedure of Waterbury et al. (1986). All removal experiments and analyses were performed using cultures in early to mid-exponential growth.

Cellular dimensions were measured using a Nikon Labophot compound microscope with a calibrated stage micrometer (n = 10 individuals). For the eleven dinoflagellates (Dinophyceae), the length corresponded to the distance from the tip of the epitheca to the hypotheca. The breadth of the cell was the distance across the widest portion of the cell, typically along the girdle or cingulum. The thickness of the cell corresponded to the distance from the dorsal to the ventral side along the cingulum. Depending on the species, the projected cross-sectional area across the girdle varied from circular to elliptical, and was calculated using the simple formula:

$$A_{(\text{cross section})} = \pi * \text{breadth} * \text{thickness} \qquad \text{(Eq. 3-1)}$$

For *Heterosigma akashiwo* (Raphidophyceae), measurements were taken along the same axes as for the dinoflagellates. The four centric diatoms (Bacillariophyceae) did not produce chains under culture conditions and remained unicellular with infrequent two-celled forms. For these cells, the length was measured along the pervalvar axis. The breadth (= thickness, due to symmetry) was measured across the perpendicular transapical axis. The projected cross-sectional area of the transapical plane was calculated using Eq. 3-1. Finally, swimming speeds for *Heterosigma akashiwo* and several dinoflagellates were obtained from published reports (Table 3-3). The swimming speed of *Heterocapsa triquetra* was taken as the averaged values from *Heterocapsa niei* (= *Cachonina niei*) which is comparable in cell dimensions.

Clay samples and preparation. The clay selected was a phosphatic clay (IMC-P2) from central Florida (IMC Phosphates, Inc.). This sample is the unused portion of phosphate ore (about 1/3 of the mass) which contains particles ≤ 125 μm, although >70% the particles are in the size range of silt and clays (Barwood, 1982). The most important minerals include the following in decreasing amounts: smectite, carbonate-fluorapatite, palygorskite, mica, interstratified clays, kaolinite, quartz, wavellite, crandallite, dolomite, calcite, feldspar, millisite, and trace amounts of heavy metals (Bromwell, 1982). The freshwater stock suspension contains 16.7% solid content (m/m). Clay suspensions for experiments were prepared by diluting the stock to the desired concentration using distilled/deionized water.

Clay screening and comparison. Removal experiments were performed following the procedure in the previous chapter (Sengco et al., 2001). The initial cell concentration was determined from in vivo cell fluorescence calibrated against cell counts. *Synechococcus* WH8017 was enumerated according to Waterbury et al. (1986). The range of cell concentrations tested for each group of organisms was determined, in part, by two considerations: (1) the range of cell concentrations found in nature, and (2) the lower limit of detection on the fluorometer. The cell concentration for the dinoflagellates ranged from 100 to 55,000 cells ml^{-1}. Diatom concentration ranged from 3,000 to 300,000 cells ml^{-1}. *Heterosigma akashiwo* was tested from 900 to 32,000 cells ml^{-1}. *Synechococcus* W8017 was tested at 1 $x10^4$ and 2 x 10^7 cells ml^{-1}, and *Aureococcus anophagefferens* was used from 1.4 x 10^5 to 3.5 x 10^6 cells ml^{-1}. For these experiments, the final clay loadings were

Table 3-2. Algal cultures and dimensions. Clonal designations and sources: CCMP = Center for the Culture of Marine Phytoplankton, Bigelow, ME and WHOI = D.M. Anderson laboratory, Woods Hole Oceanographic Institution, Woods Hole, MA. Cell dimensions were measured using a calibrated stage micrometer (Nikon light microscope). Values in parenthesis represents standard deviation, n = 10 cells. The cross-sectional area around the girdle was caluclated using both the breadth and the thickness of the cell. The final column contains the values for effective cell diameter used to calculate the collision frequency coefficient.

Class	Species	Clonal Designation or Source	length (μm)	breadth (μm)	thickness (μm)	cross-sectional area (μm²)	effective cell diameter for β calculation
Cyanophyceae	*Synechococcus WH8017*	WH8017, J. Waterbury	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.8	1.0
Chrysophyceae	*Aureococcus anophagefferens*	BP3B, E. Cosper	2.1 (0.1)	2.1 (0.1)	2.1 (0.1)	3.5	2.1
Raphidophyceae	*Heterosigma akashiwo*	CCMP452	17.8 (2.2)	6.8 (0.7)	9.0 (0.0)	47.7	9.0
Bacillariophyceae	*Skeletonema costatum*	WHOI, A. Waite	7.3 (0.8)	4.5 (0.6)	4.5 (0.6)	15.9	4.5
	Thalassiosira weisflogii	Actin, Guillard	15.5 (2.4)	12.9 (0.6)	12.9 (0.6)	130.2	12.9
	Chaetoceros gracilis	WHOI, A. Waite	8.0 (0.9)	5.9 (0.8)	5.9 (0.8)	27.1	5.9
Dinophyceae (athecate)	*Karenia brevis*	CCMP718	23.4 (2.5)	26.4 (1.2)	12.7 (1.3)	263.1	26.4
	Akashiwo sanguinea	GSBL	54.9 (3.8)	43.5 (3.5)	31.4 (1.6)	1071.9	43.5
	Gymnodinium instriatum	GIAL 177	46.0 (4.5)	34.9 (3.1)	29.8 (1.4)	814.9	34.9
	Karenia mikimotoi	Hong Kong University, D. Au	29.1 (2.1)	26.0 (2.1)	18.0 (2.1)	367.6	26.0
	Gyrodinium galatheanum	Horn Point Laboratory, A. Li	14.9 (2.4)	11.9 (0.9)	12.2 (0.6)	113.9	12.2
	Amphidinium carterae	Amphi, Guillard	16.5 (1.5)	10.4 (1.0)	9.1 (0.8)	74.4	10.4
Dinophyceae (thecate)	*Alexandrium tamarensis*	GTCA28	31.7 (1.9)	31.5 (1.8)	28.8 (3.5)	711.3	31.5
	Heterocapsa triquetra	WHOI, K. Olli	22.2 (1.9)	16.4 (1.8)	14.4 (1.2)	184.9	16.4
	Prorocentrum micans	CCMP21	45.4 (2.4)	24.8 (0.8)	17.7 (1.9)	343.9	24.8
	Prorocentrum minimum	CCMP1329	18.9 (1.1)	16.9 (1.4)	9.9 (1.4)	130.9	16.9
	Scrippsiella trochoidea	SA 2	28.5 (2.1)	21.8 (1.3)	19.0 (2.1)	324.6	21.8

Table 3-3. Algal swimming speeds. Values are averages of results from studies conducted by Kamykowski and colleagues. *Prorocentrum minimum* (= *Prorocentrum mariae-labouriae*) in Kamykowski et al., 1992.

Species	swimming speed (mm/s)	Reference
Heterosigma akashiwo	0.16	Smayda, 1998
Karenia brevis	0.28	Kamykowski, unpublished data
Akashiwo sanguinea	0.14	Kamykowski et al., 1992
Amphidinium carterae	0.24	Kamykowski and McCollum, 1986
Heterocapsa niei	0.21	Kamykowski and McCollum, 1986; Kamykowski et al., 1989
Prorocentrum micans	0.14	Kamykowski and McCollum, 1986
Prorocentrum minimum	0.17	Kamykowski et al., 1992
Scrippsiella trochoidea	0.15	Kanykowski et al., 1992

0 (distilled/deionized water), 0.03, 0.10, 0.25, 0.50 g l^{-1}. Later, when members of the same algal classes or of comparable sizes were compared at a prescribed clay loading, these were done at the following cell concentrations: the Dinophyceae (800 - 1300 cells ml^{-1}) together with *H. akashiwo* (9,300 cells ml^{-1}), the Bacillariophyceae (3,000 - 8,000 cells ml^{-1}), and finally, *Synechococcus* WH8017 (40,000 cells ml^{-1}) together with *A. anophagefferens* (300,000 cells ml^{-1}).

Collision coefficients. The collision of particles is generally a physical process controlled by the hydrodynamics of the system and external forces such as gravity. The three mechanisms that bring particles together are Brownian diffusion, velocity gradients and differential sedimentation (O'Melia and Tiller, 1996) (Table 3-1). In the current experimental design, aggregation takes place in a quiescent environment which limits collisions due to velocity gradients. Therefore, only diffusion and differential sedimentation were considered. In addition, collisions can be promoted by the motility of the algal cells (*Heterosigma akashiwo* and the dinoflagellates). This has been parameterized by Jackson and Lochmann (1996) (Table 3-1).

For this study, the collision between the individual organisms and the clays has been calculated for a range of clay particle sizes. In considering the diameter of motile species, the following assumptions were made: the direction of swimming occurs along the longtitudinal axis and the cell spins about this axis. Therefore, the largest transverse cross-sectionl area swept by the cell has a diameter equal to the longer of the cells' breadth or thickness. This effective cell diameter was used in the calculation (Table 3-2).

Mixed species. To determine whether the observed difference in removal patterns between *Karenia brevis* and other microalgae when the species are in the same medium, *K. brevis*, at constant concentration, was mixed with two different concentrations of a dinoflagellate (*Prorocentrum micans*) and a diatom (*Skeletonema costatum*). The organisms were cultured separately and then combined. In the first experiment, ca. 1000 cell ml^{-1} of *K. brevis* was mixed with either 400 cell ml^{-1} (low) or 1600 cell ml^{-1} (high) of *P. micans*. The mixed cultures were placed in duplicate borosilicate test tubes and were treated with 0, 0.03, 0.10, 0.25, and 0.50 g L^{-1} of IMC-P2 Florida phosphatic clays as previously described. The initial and final cell counts were performed on 2-ml preserved samples (Utermohl) using a Nikon light microscope with a Sedgewick-Rafter counting

chamber. In the second experiment, ca. 3000 cell ml^{-1} of *K. brevis* was mixed with either 35,000 cell ml^{-1} (low) an 300,000 cell ml^{-1} (high) of *Skeletonema costatum.* Due to the difference in size and numbers, the cell counts were done twice using a Sedgewick-Rafter chamber to enumerate the *K. brevis* cells and a Fuchs-Rosenthal counting chamber for the diatom.

Field mesocosm experiments. The mesocosms consisted of four fiberglass tanks (1 m across, 0.725 m high, volume 530 L). The tanks were set up adjacent to the Texas State Aquarium in Corpus Christi, Texas. Natural, *Karenia*-rich water was pumped from the aquarium dock directly into the tanks using a diaphragm pump. The phosphatic clay (dry weight was about 77%) was soaked in seawater after a few hours and then broken up with a kitchen blender to produce a slurry in 20 L of seawater. Clay loading was 0.05 g L^{-1}. The clay slurry was dispersed over two replicate tanks using a pair of submersible pumps. Unfiltered seawater was added to the control tanks. Aggregation was allowed to proceed for 2.5 hours without agitation or mixing. Before and after clay dispersal, integrated samples of water were taken by lowering silicone tubes in two locations in the tank (i.e. within-tank replication). 200 mL each were collected at the surface, at 0.25 m and at 0.5 m below the surface. The samples were pooled into acid-washed 2-L, polycarbonate bottles. 50 mL subsamples were preserved in Lugol's solution for *K. brevis* and total community cell counts. For the samples before clay addition, 20 mL from each of the pairs of subsamples within each tank were combined (40 mL total). The volume was allowed to settle for 24 hours in a Utermohl settling column. For the samples after clay addition, 25 mL were combined and settled. All samples were counted on a Zeiss IM35 inverted microscope. Only the species with more than 300 individuals in the samples were considered in this study. Statistical analysis was done using SigmaStat (SPSS, Inc).

Results

Removal efficiency and cell concentration effects. For all organisms, the RE increased with clay loading (Figures 3-1, 3-2, 3-3, and 3-4). In most cases, the curves for different loadings were parallel to one another but shifted to higher removal values as the clay loading increased. At 0.25 and 0.50 g L^{-1}, the removal efficiency of several species approached a maximum value: *Karenia brevis* (Fig. 3-1A), *Akashiwo sanguinea* (Fig. 3-

Figure 3-1. Removal efficiency using IMC-P2 phosphatic clay versus cell density of six athecate dinoflagellates. The values in parenthesis represent the projected cross-sectional area of the organism calculated around the girdle or cingulum. Error bars are standard deviation (n = 3). Clay loading were 0.03 g L^{-1} (◆), 0.10 g L^{-1} (■), 0.25 g L^{-1} (▲), and 0.50 g L^{-1} (●).

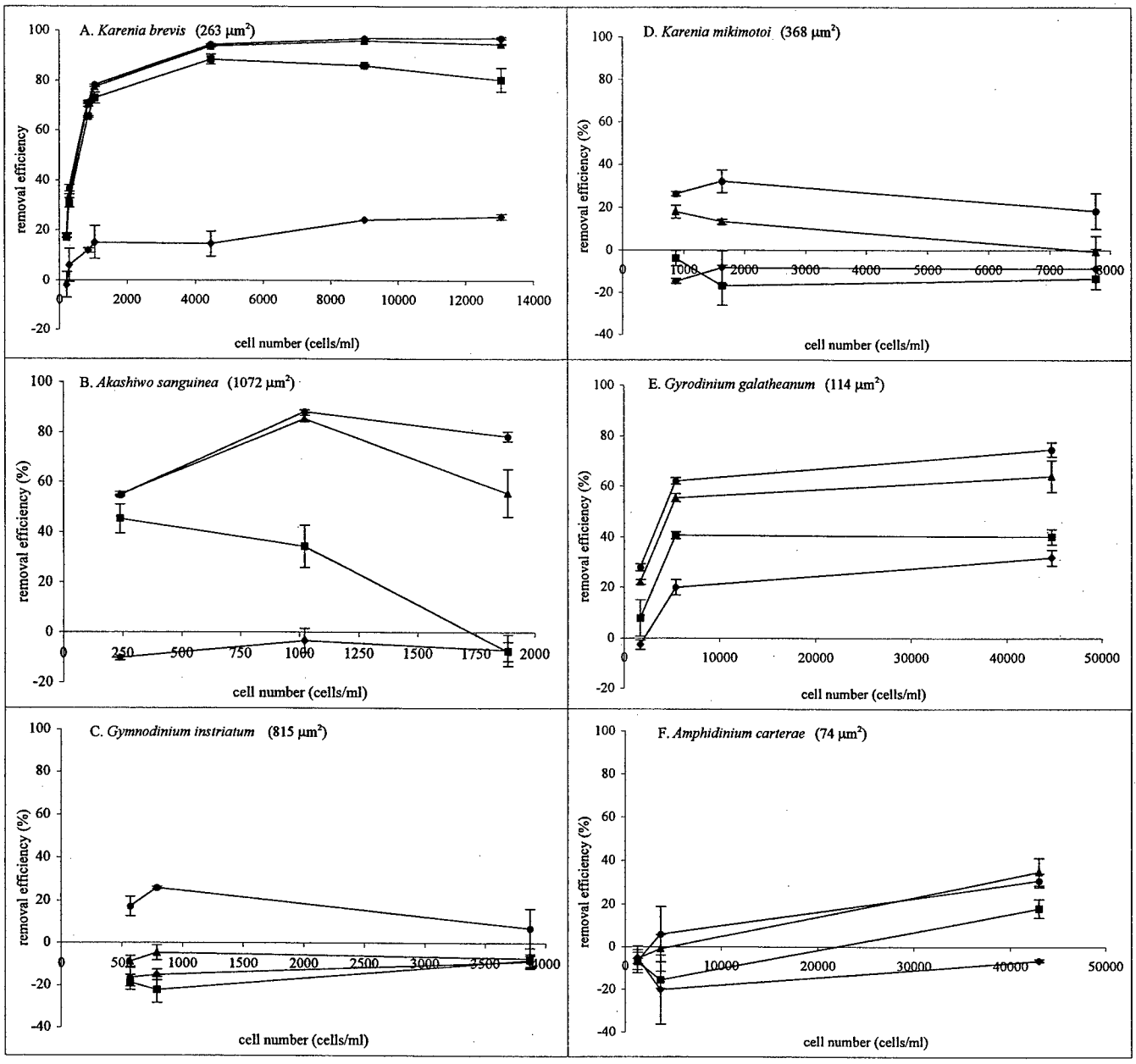

A. Karenia brevis (263 µm²)
removal efficiency
cell number (cells/ml)
D. Karenia mikimotoi (368 µm²)
removal efficiency (%)
cell number (cells/ml)
B. Akashiwo sanguinea (1072 µm²)
removal efficiency (%)
cell number (cells/ml)
E. Gyrodinium galatheanum (114 µm²)
removal efficiency (%)
cell number (cells/ml)
C. Gymnodinium instriatum (815 µm²)
removal efficiency (%)
cell number (cells/ml)
F. Amphidinium carterae (74 µm²)
removal efficiency (%)
cell number (cells/ml)

Figure 3-2. Removal efficiency using IMC-P2 phosphatic clay versus cell density of five thecate dinoflagellates and the raphidophyte *Heterosigma akashiwo*. The values in parenthesis represent the projected cross-sectional area of the organism calculated around the girdle or cingulum for the dinoflagellates. Error bars are standard deviation (n = 3). Clay loading were 0.03 g L^{-1} (◆), 0.10 g L^{-1} (■), 0.25 g L^{-1} (▲), and 0.50 g L^{-1} (●).

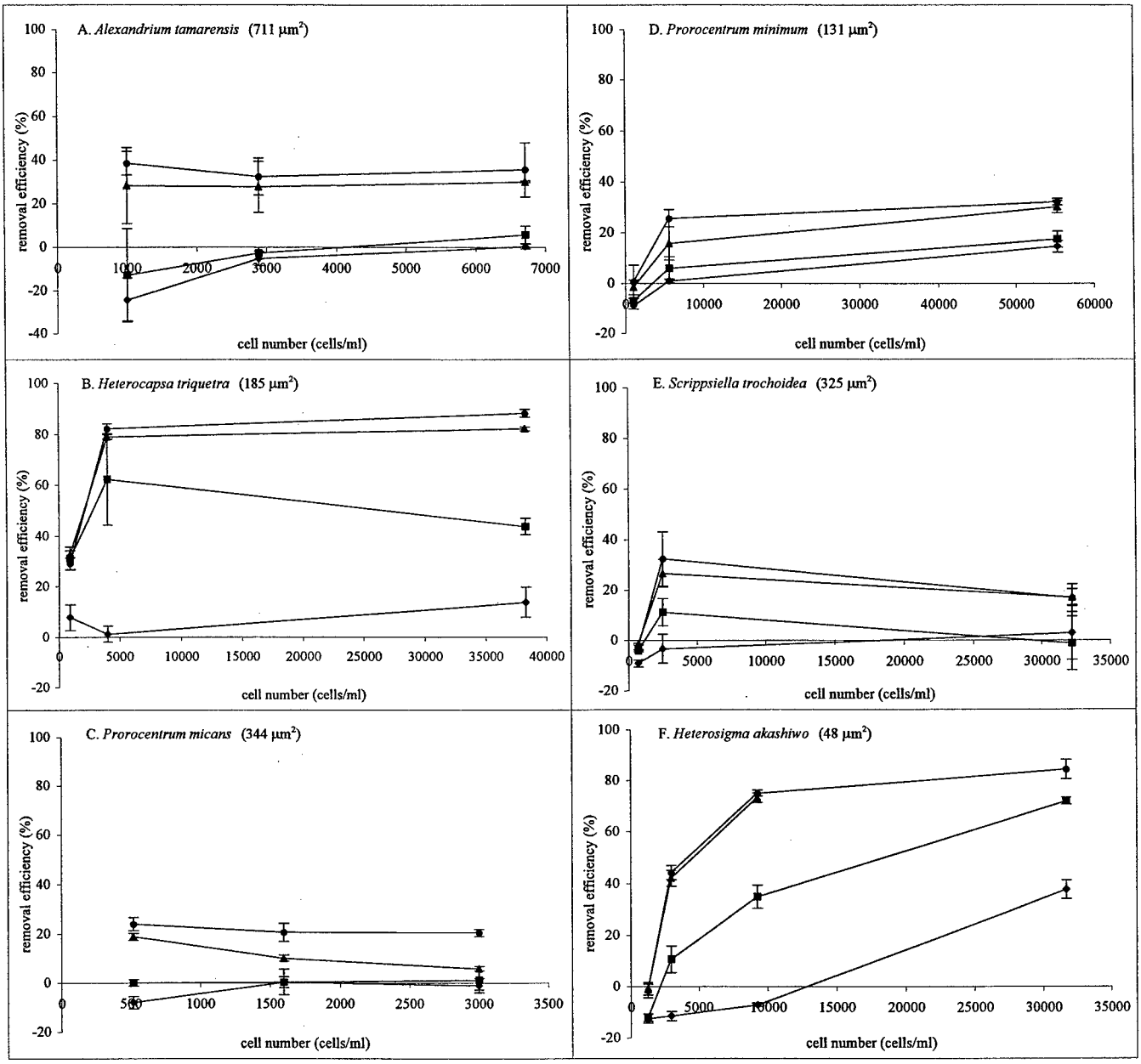

A. Alexandrium tamarensis (711 µm²)
D. Prorocentrum minimum (131 µm²)
B. Heterocapsa triquetra (185 µm²)
E. Scrippsiella trochoidea (325 µm²)
C. Prorocentrum micans (344 µm²)
F. Heterosigma akashiwo (48 µm²)
removal efficiency (%)
cell number (cells/ml)

Figure 3-3. Removal efficiency using IMC-P2 phosphatic clay versus cell density of three diatoms. The values in parenthesis represent the projected cross-sectional area of the organism calculated for the transapical plane. Error bars are standard deviation (n = 3). Clay loading were 0.03 g L^{-1} (◆), 0.10 g L^{-1} (■), 0.25 g L^{-1} (▲), and 0.50 g L^{-1} (●).

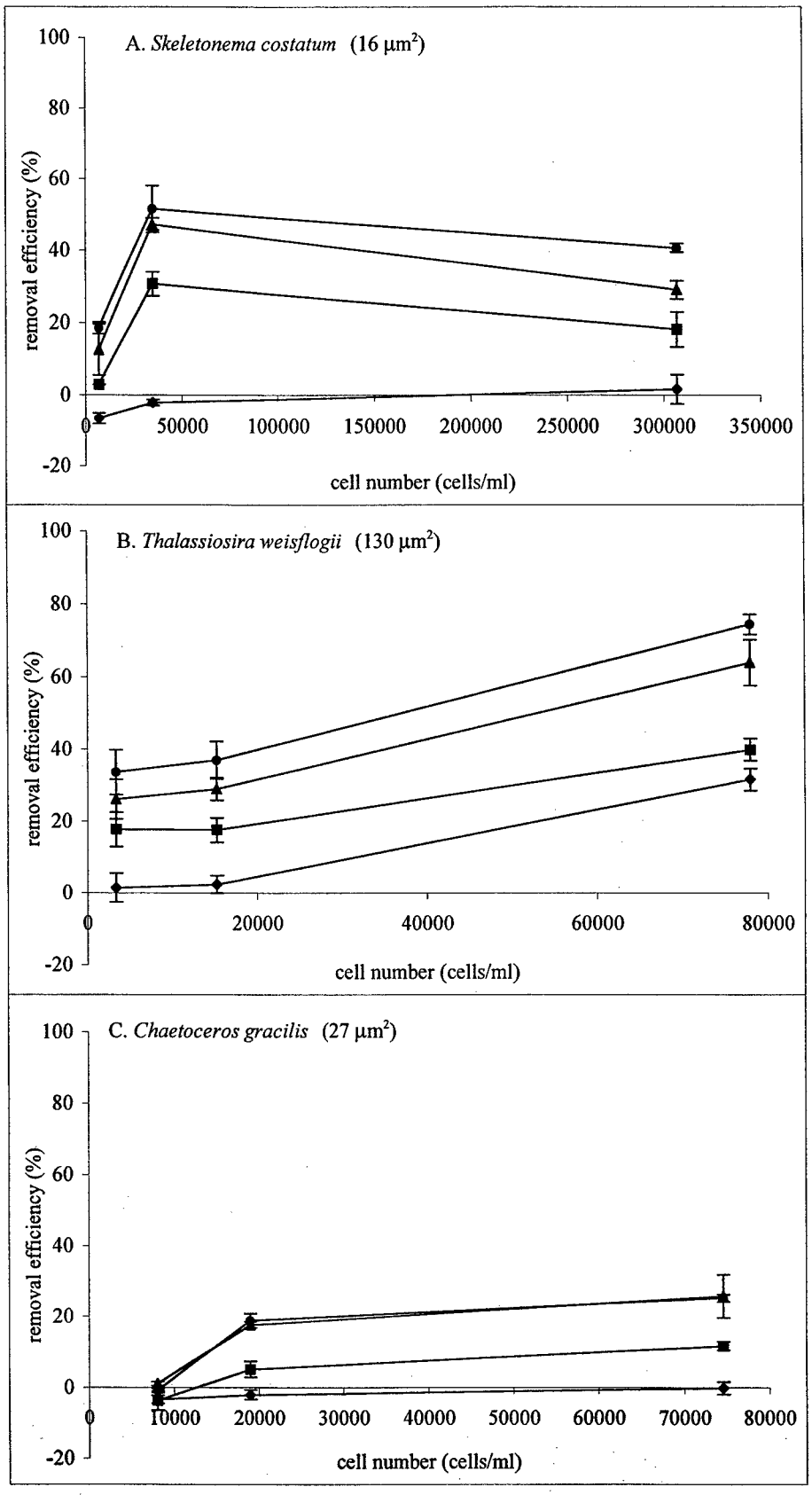
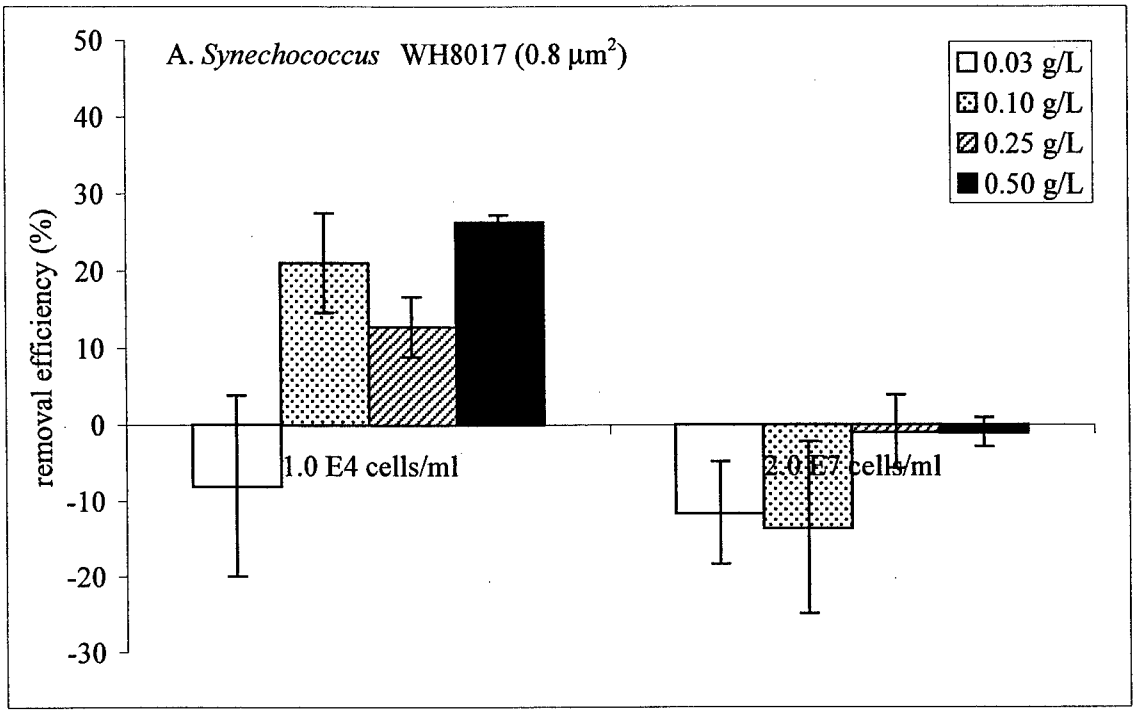
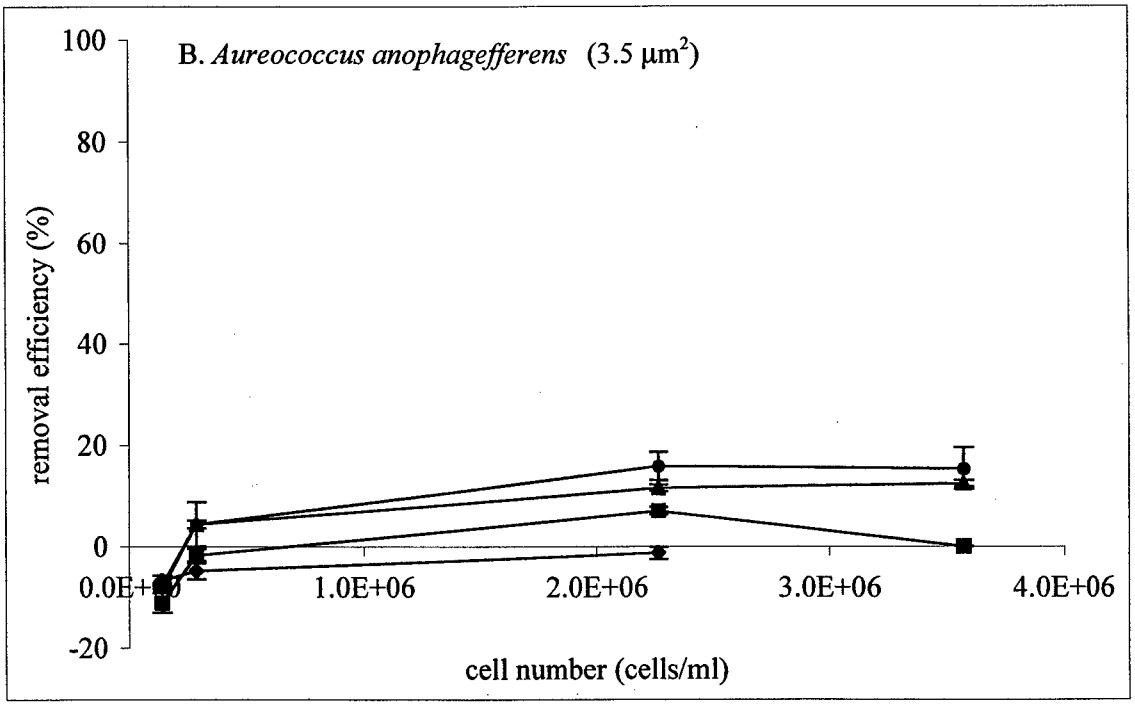

A. Skeletonema costatum (16 µm²)
removal efficiency (%)
100
80
60
40
20
0
-20
0
50000
100000
150000
200000
250000
300000
350000
cell number (cells/ml)
B. Thalassiosira weisflogii (130 µm²)
removal efficiency (%)
100
80
60
40
20
0
-20
0
20000
40000
60000
80000
cell number (cells/ml)
C. Chaetoceros gracilis (27 µm²)
removal efficiency (%)
100
80
60
40
20
0
-20
0
10000
20000
30000
40000
50000
60000
70000
80000
cell number (cells/ml)

Figure 3-4. Removal efficiency using IMC-P2 phosphatic clay versus cell density of the cyanobacterium *Synechococcus* WH8017 and chrysophyte *Aureococcus anophagefferens*. The values in parenthesis represent the projected cross-sectional area of the organism. Error bars are standard deviation (n = 3). Clay loading were 0.03 g L^{-1} (◆), 0.10 g L^{-1} (■), 0.25 g L^{-1} (▲), and 0.50 g L^{-1} (●).


A. Synechococcus WH8017 (0.8 µm²)
0.03 g/L
0.10 g/L
0.25 g/L
0.50 g/L
removal efficiency (%)
50
40
30
20
10
0
-10
-20
-30
1.0 E4 cells/ml
2.0 E7 cells/ml


B. Aureococcus anophagefferens (3.5 µm²)
removal efficiency (%)
100
80
60
40
20
0
-20
0.0E+00
1.0E+06
2.0E+06
3.0E+06
4.0E+06
cell number (cells/ml)

1B), *Amphidinium carterae* (Fig.3-1 F), *Alexandrium tamarensis* (Fig. 3-2A), *Heterocapsa triquetra* (Fig. 3-2B), *Prorocentrum minimum* (Fig. 3-2D), *Scrippsiella trochoidea* (Fig. 3-2E), *Heterosigma akashiwo* (Fig. 3-2F), *Chaetoceros gracilis* (Fig. 3-3C) and *Aureococcus anophagefferens* (Fig. 3-4B).

However, the relationship between cell concentration and removal efficiency displayed different trends among species. In 9 of 17 organisms, the removal efficiency increased as cell concentration increased: *K. brevis, Akashiwo sanguinea, Heterocapsa triquetra, Heterosigma akashiwo, Gyrodinium galatheanum* (Fig. 3-1E), *Amphidinium carterae, Prorocentrum, minimum, Thalassiosira weisflogii* (Fig. 3-3B) and *Chaetoceros gracilis* (Fig. 3-3C). In addition, the first four species listed yielded the highest removal values (>78%) using Florida phosphatic clay at 0.25 g L^{-1}. *K. brevis* and *A. sanguinea* attained this maximum value at 1000 cells ml^{-1}. *Heterocapsa triquetra* required cell concentrations greater than 5000 cells ml^{-1} to achieve the maximum, while *Heterosigma akashiwo* needed 10,000 cell ml^{-1}. Also in this group, *Gyrodinium galatheanum* and *Thalassiosira weisflogii* reached 60% removal efficiency at 0.50 g L^{-1} clay loading at 50,000 and 78,000 cells ml^{-1}, respectively. The remaining three species listed and the following eight organisms below did not exceed 40% removal efficiency at any clay loading or cell concentration.

In four species, the removal efficiency increased initially as cell concentration increased but then declined, even as clay loading increased: *Gymnodinium instriatum* (Fig. 3-1C), *Scrippsiella trochoidea, Skeletonema costatum*, and *Aureococcus anophagefferens*. In the four remaining species, the removal efficiency was either unchanged or decreased with cell concentration: *Gymnodinium mikimotoi* (Fig. 3-1D), *Alexandrium tamarensis* (Fig. 3-2A), *Prorocentrum micans* (Fig. 3-2C) and *Synechococcus* WH8017 (Fig. 3-4A).

In almost all cases, negative removal efficiency (i.e. a greater number of cells in suspension with clay treatment relative to the untreated control after 2.5 hours) was observed. It was most prevalent at the combination of low clay loading (0.03 g L^{-1}) and low cell concentration. During the experiment, a diffuse, turbid layer of fine particles was seen at the surface of the tubes even after the 2.5 hour incubation. The layer appeared stable and did not disperse or sink. Organisms may have been caught in this layer and

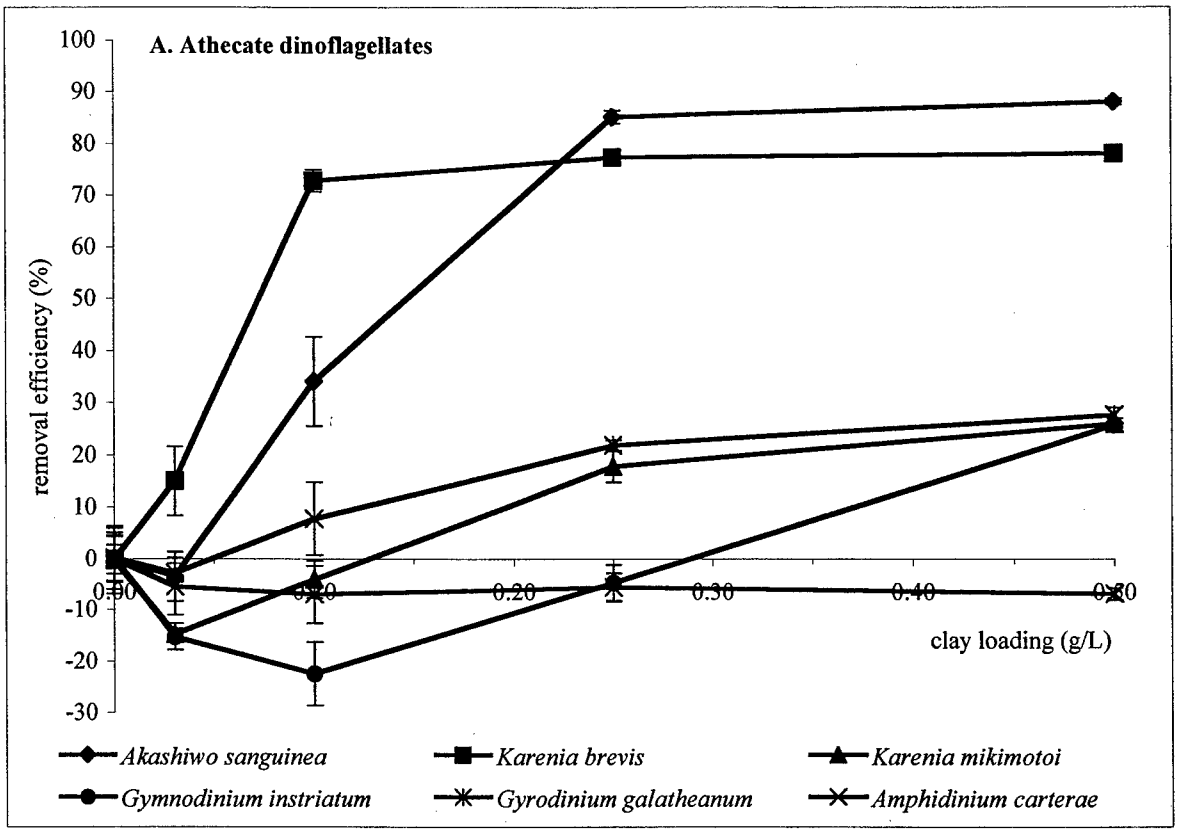
thus were prevented from sinking away from the supernatant. In the controls, however, the absence of the layer provided no means of keeping the cells in suspension. In several instances, the negative removal efficiency was more prominent at the low clay loadings (i.e. 0.03 - 0.10 g L^{-1}), even as the number of cells increased: *Akashiwo sanguinea*, *Gymnodinium instriatum*, *Gymnodinium mikimotoi*, *Amphidinium carterae*, *Alexandrium tamarensis*, *Prorocentrum micans*, *Scrippsiella trochoidea*, *Skeletonema costatum*, *Chaetoceros gracilis*, *Synechococcus* WH8017, and *Aureococcus anophagefferens*. *Gymnodinium instriatum* (Fig. 3-1C) showed mostly negative values up to 0.25 g L^{-1} of clay.

Removal efficiency with algal taxonomy, size and swimming speed. Algae grouped according to class and removal efficiency were compared at relatively similar cell concentrations (Figures 3-5, 3-6 and 3-7). The values chosen were within the range of concentrations that would be found in a natural bloom in the field based on published reports.

For the dinoflagellates and the motile raphidophyte, *Heterosigma akashiwo*, the removal patterns at ca. 1000 cell ml^{-1} were highly variable. Both *Karenia brevis* and *Akashiwo sanguinea* reached > 70 % removal, with *K. brevis* attaining this value when cell loading was 0.10 g L^{-1}, and *A. sanguinea* when clay loading was > 0.20 g l^{-1} (Fig. 3-5A). The remaining athecate dinoflagellates, the thecate dinoflagellates and *H. akashiwo* were all below 35% removal at this cell concentration (Figure 3-5 A and B). *Heterocapsa triquetra* was the first to reach the 30% mark at 0.10 g L^{-1} of phosphatic clay. The maximum removal of the three diatom species (at 3,000-8,000 cells ml^{-1}), the cyanobacterium *Synechococcus* WH8017 (at 40,000 cells ml^{-1}), and the chrysophyte *Aureococcus anophagefferens* (300,000 cells ml^{-1}) did not exceed 30% removal efficiency (Figures 3-6 and 3-7). The removal patterns of the diatoms with silica frustules were not different from the thecate dinoflagellates.

There was a very poor correlation between removal efficiency and cell length (R^2 = 0.15, Appendix A-4) and the projected cross-sectional area of the various algal species (Figure 3-8A, R^2 = 0.23). *Akashiwo sanguinea* (1072 μm^2), the largest dinoflagellate tested, displayed > 77% removal efficiency. However, the next largest species, *Gyrodinium instriatum* (815 μm^2) and *Alexandrium tamarensis* (711 μm^2) were removed

Figure 3-5. Removal efficiency of dinoflagellates and the raphidophyte *Heterosigma akashiwo* versus loadings of the IMC-P2 phosphatic clay. Dinoflagellate cell number ranged from 800 to 1300 cells ml^{-1}. *Heterosigma akashiwo* was tested at 9,300 cells ml^{-1}. (A) Athecate dinoflagellates. (B) Thecate dinoflagellates and the raphidophyte *Heterosigma akashiwo*. Error bars represent standard deviation (n = 3).


A. Athecate dinoflagellates
removal efficiency (%)
clay loading (g/L)
Akashiwo sanguinea
Karenia brevis
Karenia mikimotoi
Gymnodinium instriatum
Gyrodinium galatheanum
Amphidinium carterae

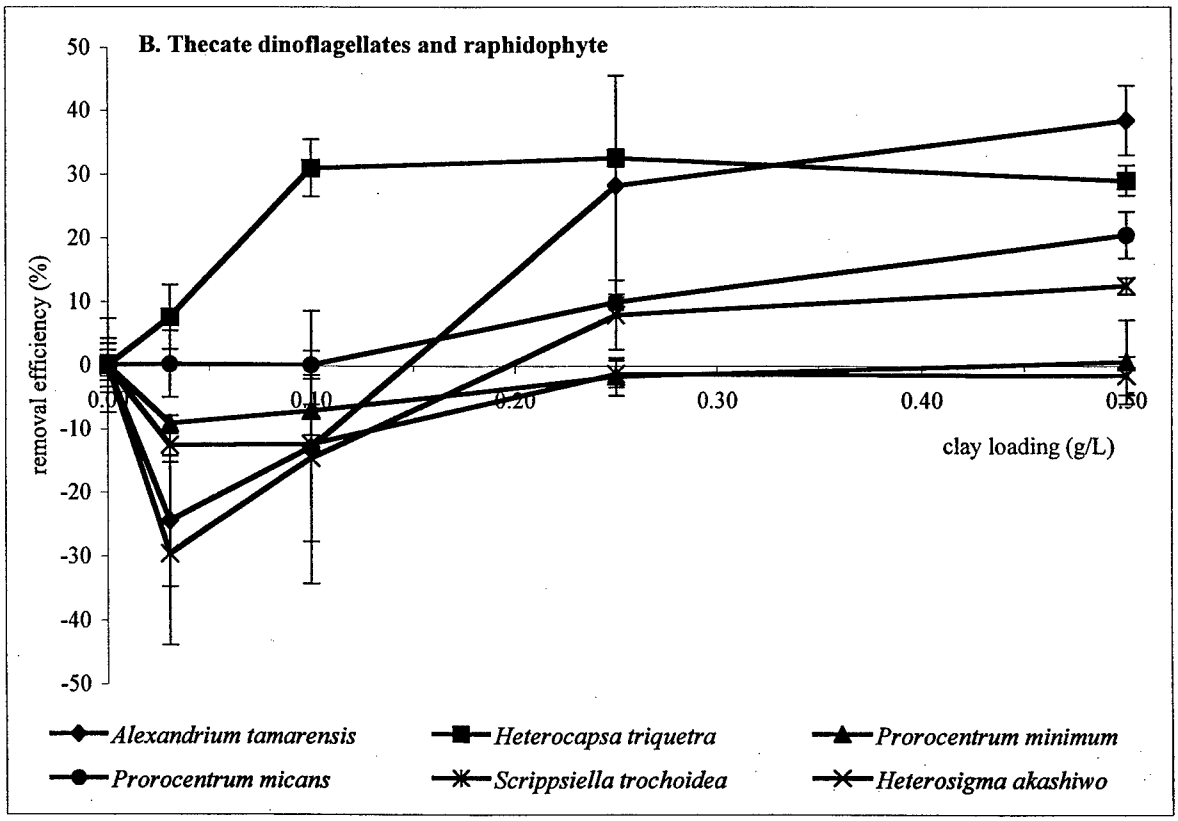

B. Thecate dinoflagellates and raphidophyte
removal efficiency (%)
clay loading (g/L)
Alexandrium tamarensis
Heterocapsa triquetra
Prorocentrum minimum
Prorocentrum micans
Scrippsiella trochoidea
Heterosigma akashiwo

Figure 3-6. Removal efficiency of marine diatoms versus the loadings of IMC-P2 phosphatic clay. Diatom concentration ranged from 3,000 to 8,000 cells ml^{-1}. Error bars represent standard deviation (n = 3).

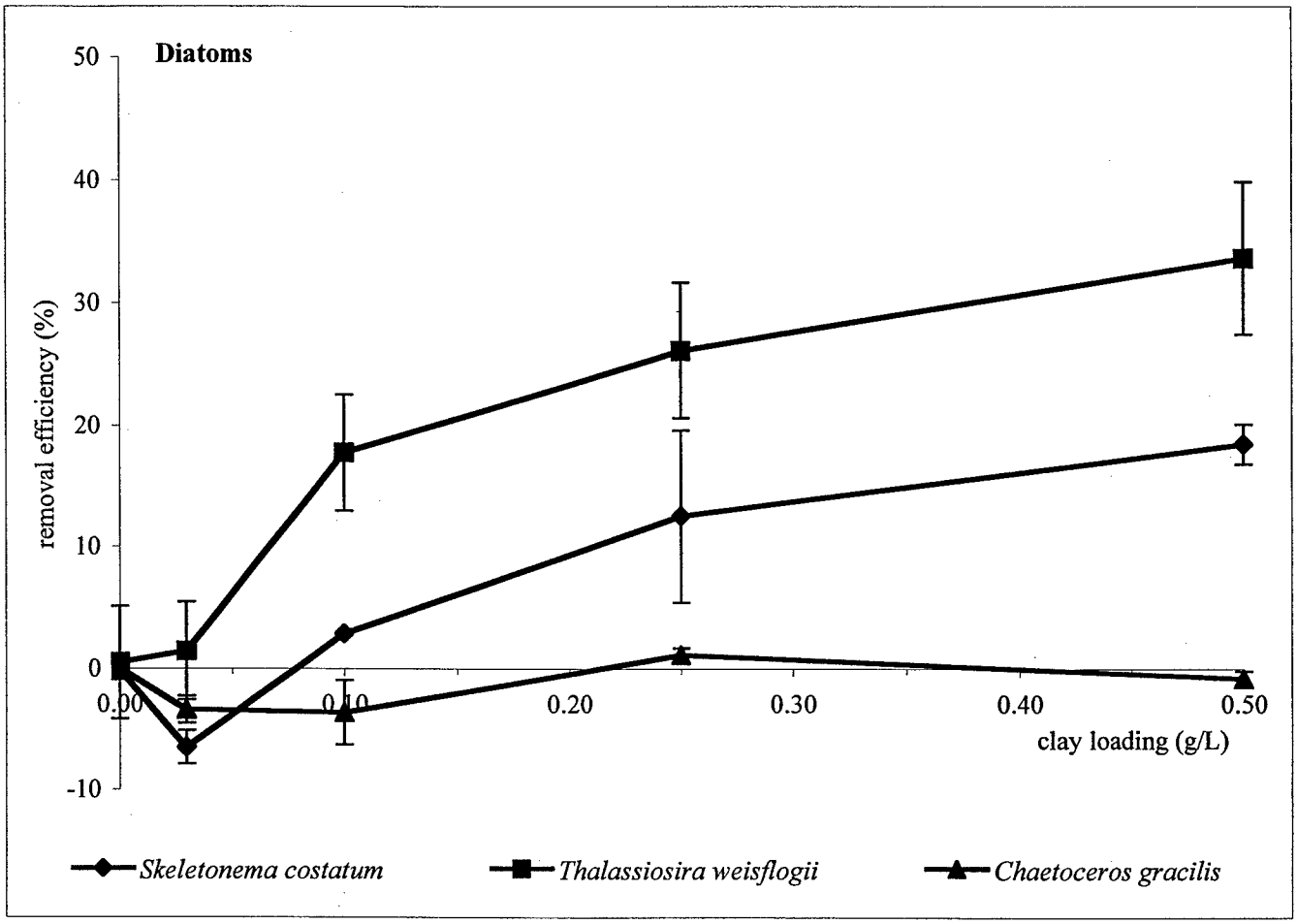

Diatoms
50
40
30
20
10
0
-10
removal efficiency (%)
0.00
0.10
0.20
0.30
0.40
0.50
clay loading (g/L)
Skeletonema costatum
Thalassiosira weisflogii
Chaetoceros gracilis

Figure 3-7. Removal efficiency of the marine cyanobacterium *Synechococcus* WH8017 and the chrysophyte *Aureococcus anophagefferens* versus the loadings of IMC-P2 phosphatic clay. *Synechococcus* WH8017 was tested at 40,000 cells ml^{-1} and *Aureococcus anophagefferens* at 300,000 cells ml^{-1}. Error bars represent standard deviation (n = 3).

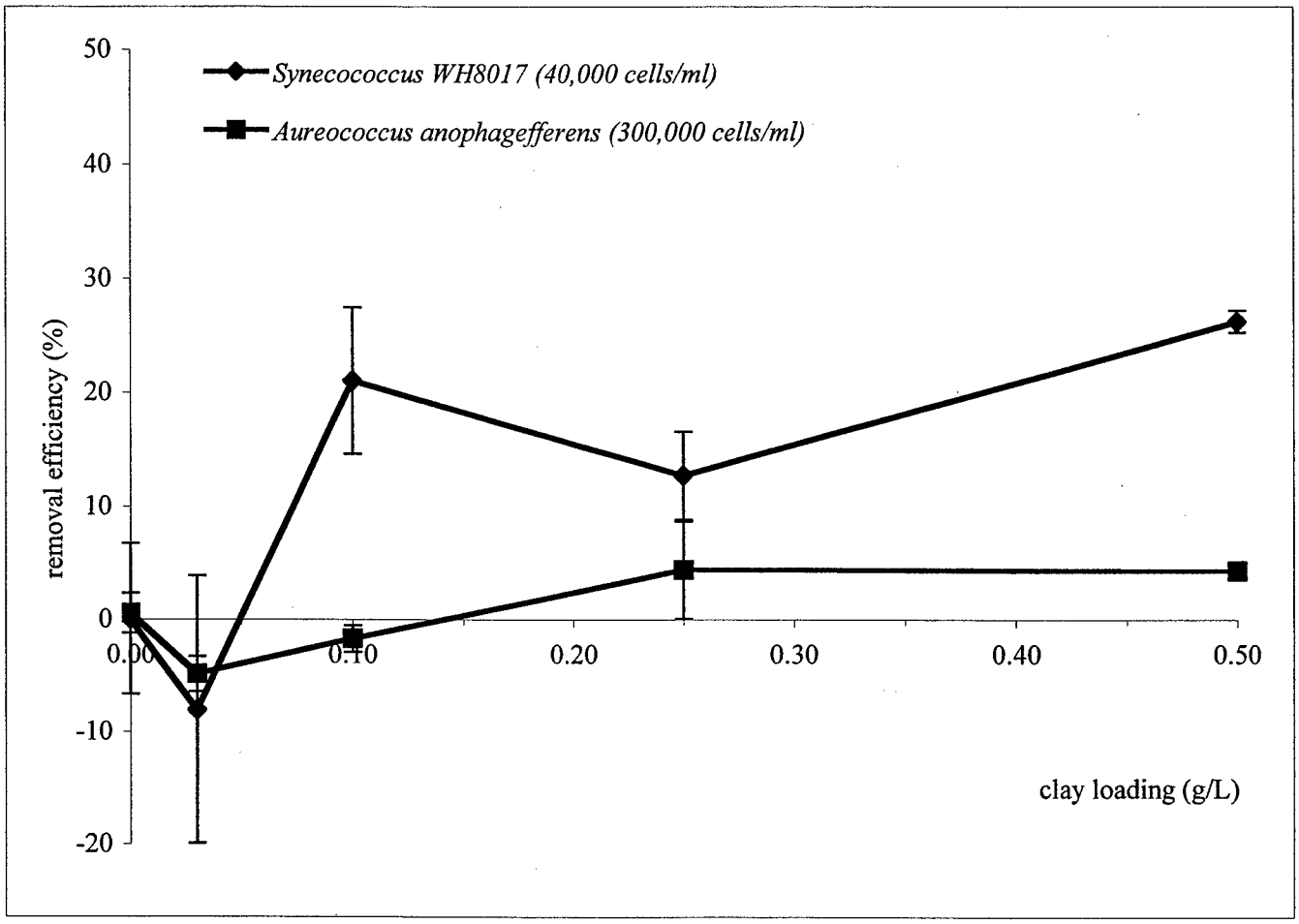
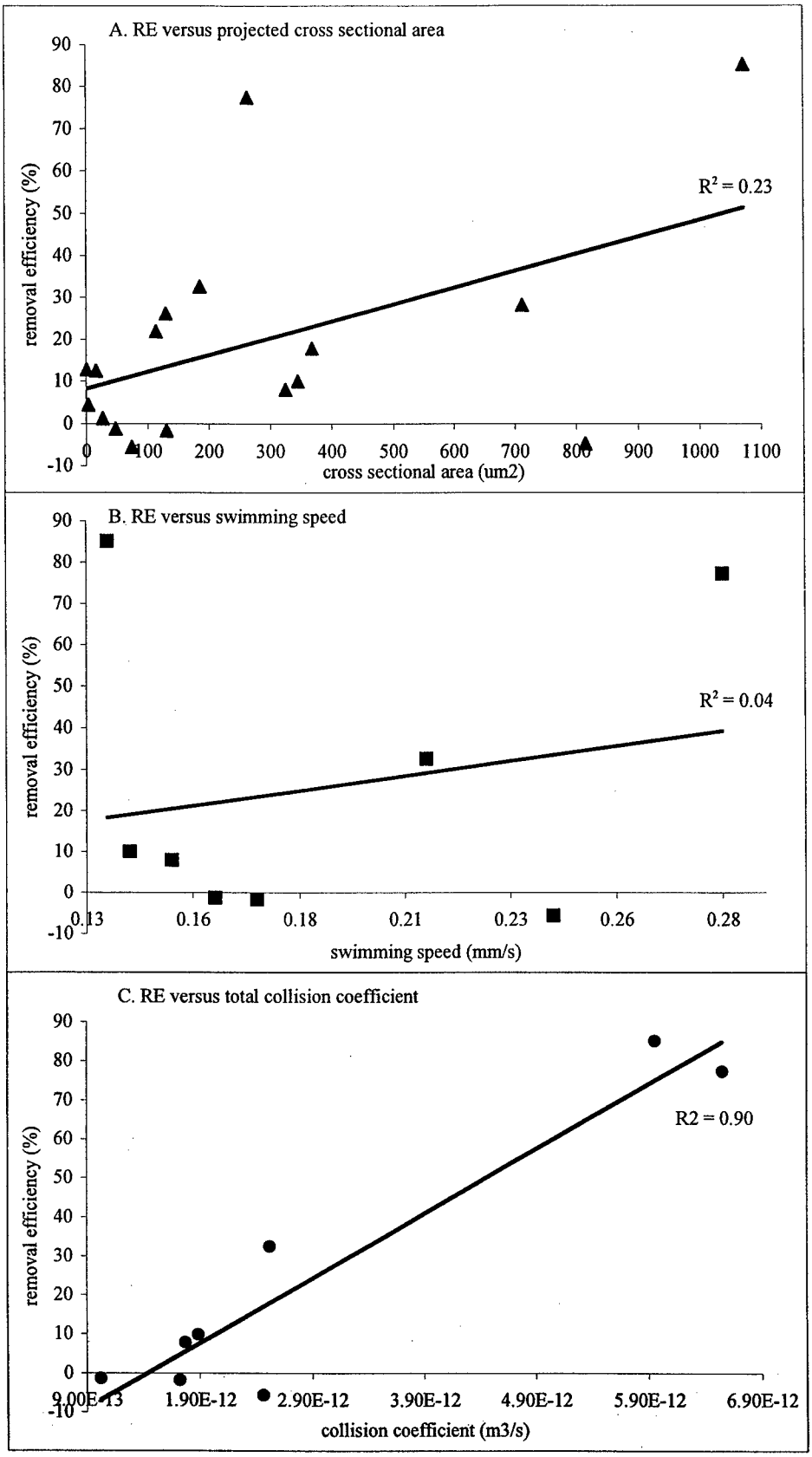

Synecococcus WH8017 (40,000 cells/ml)
Aureococcus anophagefferens (300,000 cells/ml)
removal efficiency (%)
50
40
30
20
10
0
-10
-20
0.00
0.10
0.20
0.30
0.40
0.50
clay loading (g/L)

Figure 3-8. Comparison of removal efficiency with geometric or dynamic characteristics of algal species studied. (A) Projected cross-sectional area, (B) Swimming speed of flagellates, (C) Total collision frequency coefficient of flagellates at clay size = 10 μm. The algal diameters used in the calculation of collision frequency coefficient are listed in Table 3-2. The linear regression was plotted along with the R-squared value.


A. RE versus projected cross sectional area
removal efficiency (%)
cross sectional area (um2)
R² = 0.23
B. RE versus swimming speed
removal efficiency (%)
swimming speed (mm/s)
R² = 0.04
C. RE versus total collision coefficient
removal efficiency (%)
collision coefficient (m3/s)
R2 = 0.90

at < 40%. Despite having relatively similar sizes and shapes, *Karenia brevis* (263 μm^2, > 80 % removal) and *Gymnodinium mikimotoi* (367 μm^2, < 25 % removal) were affected differently, as were similarly-sized *Prorocentrum micans* (345 μm^2) and *Scrippsiella trochoidea* (325 μm^2), both removed at < 40 %. For the smaller dinoflagellate cells, the RE of *Heterocapsa triquetra* (185 μm^2) was higher than the two smaller but generally comparable species, *Prorocentrum minimum* (131 μm^2) and *Gyrodinium galatheanum* (114 μm^2).

As with algal size, there was a poor relationship between RE and the swimming speed of the flagellates (Figure 3-8B, $R^2 = 0.04$). For example, the two species with high and relatively similar RE's, *K. brevis* and *A. sanguinea*, had swimming rates that differed by a factor of two (Table 3-3): 0.28 mm s^{-1} and 14 mm s^{-1}, respectively. Conversely, two cells with very similar swimming speeds, *Heterocapsa triquetra* (0.21 mm s^{-1}) and *Amphidinium carterae* (0.24 mm s^{-1}), were removed 30% and -5.5% at 0.10 g L^{-1}.

For diatoms, the RE of the largest cell, *Thalassiosira weisflogii* (130 μm^2) was the highest (Fig. 3-6). However, the smallest cell, *Skeletonema costatum* (16 μm^2) had a higher removal efficiency than the slightly larger *Chaetoceros gracilis* (27 μm^2). The smallest species in the survey, *Synechococcus* WH8017 (0.8 μm^2) was removed slightly better than *Aureococcus anophagefferens* (3.5 μm^2) (Fig. 3-7).

Removal efficiency and the collision frequency coefficient. The collision frequency coefficient for each of the transport mechanisms (i.e. diffusion, motility and differential sedimentation) was calculated for the organism and for a clay particle (Table 3-1). The calculations were performed for a range of clay particle sizes (0.01 μm to 10 mm) and a constant cell size (i.e. Table 3-2). The total collision coefficient represents the sum of the three mechanisms.

This procedure is demonstrated for *K. brevis* in Figure 3-9, which was representative of all flagellated species in the survey. According to Fig. 3-9, collisions due to Brownian diffusion were not significant (< 1.54×10^{-15} m^3 s^{-1}). However, interparticle collisions due to cell motility were important when clay particles were less than ca. 50 μm in size. Such collisions contributed to the total value more than differential sedimen-

tation. When the clay particles exceeded 50 μm, collisions between the cells and the clay particles (or aggregates) were dominated by differential sedimentation.

The collision frequency coefficient for swimming and the total coefficient were calculated and compared for all flagellate species where data on swimming speeds were available (Figure 3-10). These values considered the combined effects of cell size and swimming speed (Fig. 3-10A). Only collisions between cells and clay particles < 50 μm were compared. *Karenia brevis* showed the highest values (4.35×10^{-12} to 1.09×10^{-11} m^3 s^{-1}) immediately followed by *Akashiwo sanguinea* (1.79×10^{-12} to 3.84×10^{-12} m^3 s^{-1}). Both had the highest removal efficiencies in this survey. Among the flagellates with removal values less than 40% (Fig. 3-5A-B), *Heterocapsa triquetra* had the highest removal value, consistent with its ranking among the predicted collision coefficients (1.52×10^{-12} to 6.13×10^{-12} m^3 s^{-1}). Finally, the ranking of the total collision frequency coefficients followed values from cell motility alone (Figure 3-10B). The predicted coefficients for *K. brevis* and *A. sanguinea* became closer when the values from differential sedimentation was combined with those from motility.

For the diatoms, collision coefficients were lower than the flagellates but higher than *Synechococcus* and *Aureococcus* (Figure 3-11A). As with the flagellates, differential sedimentation was dominant over Brownian diffusion for the species. There were no collisions due to swimming because these species are non-motile. The order of the predicted collision coefficients for each diatom was in good agreement with the order of cell sizes (i.e. diameters) (compare Fig. 3-11B and Table 3-2): *Thalassiosira weisflogii* (2.09×10^{-14} to 6.76×10^{-12} m^3 s^{-1}) > *Chaetoceros gracilis* (1.08×10^{-15} to 5.6×10^{-12} m^3 s^{-1}) > *Skeletonema costatum* (4.5×10^{-16} to 5.4×10^{-12} m^3 s^{-1}).

Finally, *Synechococcus* WH8017 showed the lowest collision coefficient (3.6×10^{-17} to 4.76×10^{-12} m^3 s^{-1}), followed by *Aureococcus anophagefferens* (8.3×10^{-17} to 4.97×10^{-12} m^3 s^{-1}). Unlike the previous organisms, collisions due to diffusion were critical for both of these species when the clay particles were < 1 μm given their cell sizes < 2 microns). At larger particle sizes, differential sedimentation was dominant for these non-motile cocci (Fig. 3-11A and B).

Comparing all data for the motile species, the correlation between the RE and the total predicted collision frequency coefficient was better than for size alone and swim-

Figure 3-9. Collision frequency coefficients for *Karenia brevis*. Brownian diffusion, swimming motility, differential sedimentation and the sum of all three (i.e. total). The equations and variables are listed in Table 3-1. Cell diameters used in the calculation of collision frequency coefficients are listed in Table 3-2.

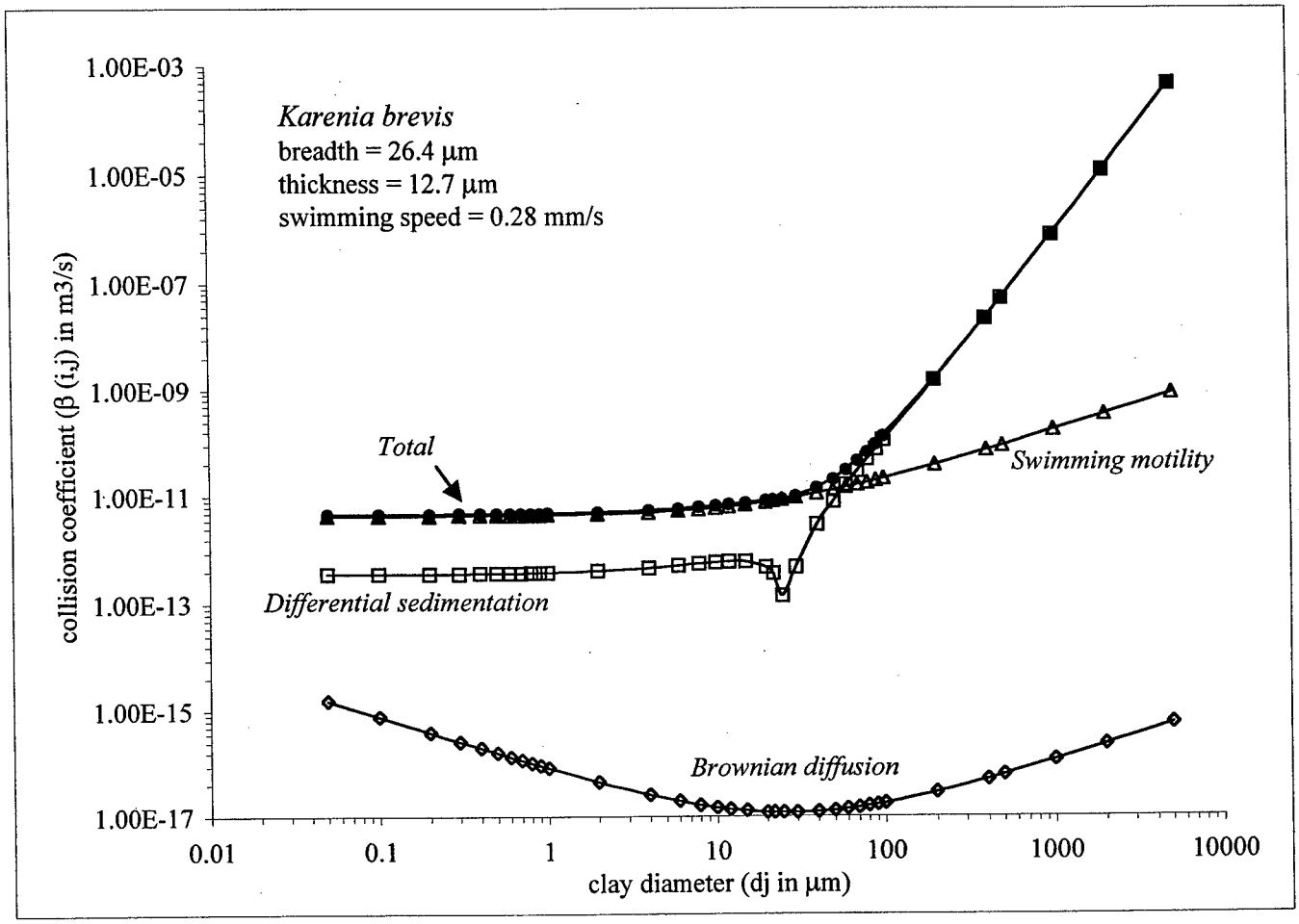
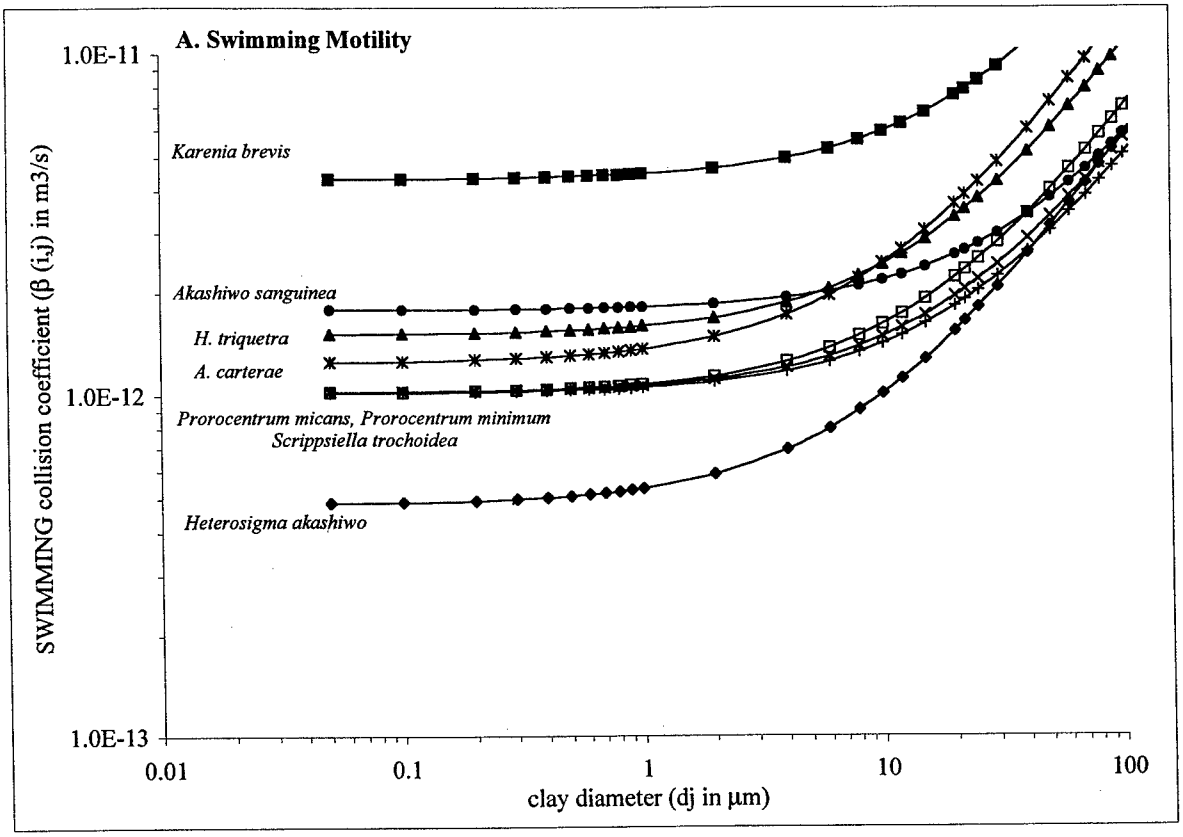

1.00E-03
1.00E-05
1.00E-07
1.00E-09
1.00E-11
1.00E-13
1.00E-15
1.00E-17
collision coefficient (β (i,j) in m3/s)
Karenia brevis
breadth = 26.4 µm
thickness = 12.7 µm
swimming speed = 0.28 mm/s
Total
Swimming motility
Differential sedimentation
Brownian diffusion
0.01
0.1
1
10
100
1000
10000
clay diameter (dj in µm)

Figure 3-10. Comparison of collision frequency coefficients among algal species assuming a range of clay diameters. (A) Collisions due to cell motility of flagellates. (B) Total collision coefficients including Brownian diffusion, cell motility and differential sedimentation for flagellates. Cell diameters used in the calculation of collision frequency coefficients are listed in Table 3-2.


A. Swimming Motility
1.0E-11
1.0E-12
1.0E-13
SWIMMING collision coefficient (β (i,j) in m3/s)
Karenia brevis
Akashiwo sanguinea
H. triquetra
A. carterae
Prorocentrum micans, Prorocentrum minimum
Scrippsiella trochoidea
Heterosigma akashiwo
0.01
0.1
1
10
100
clay diameter (dj in µm)

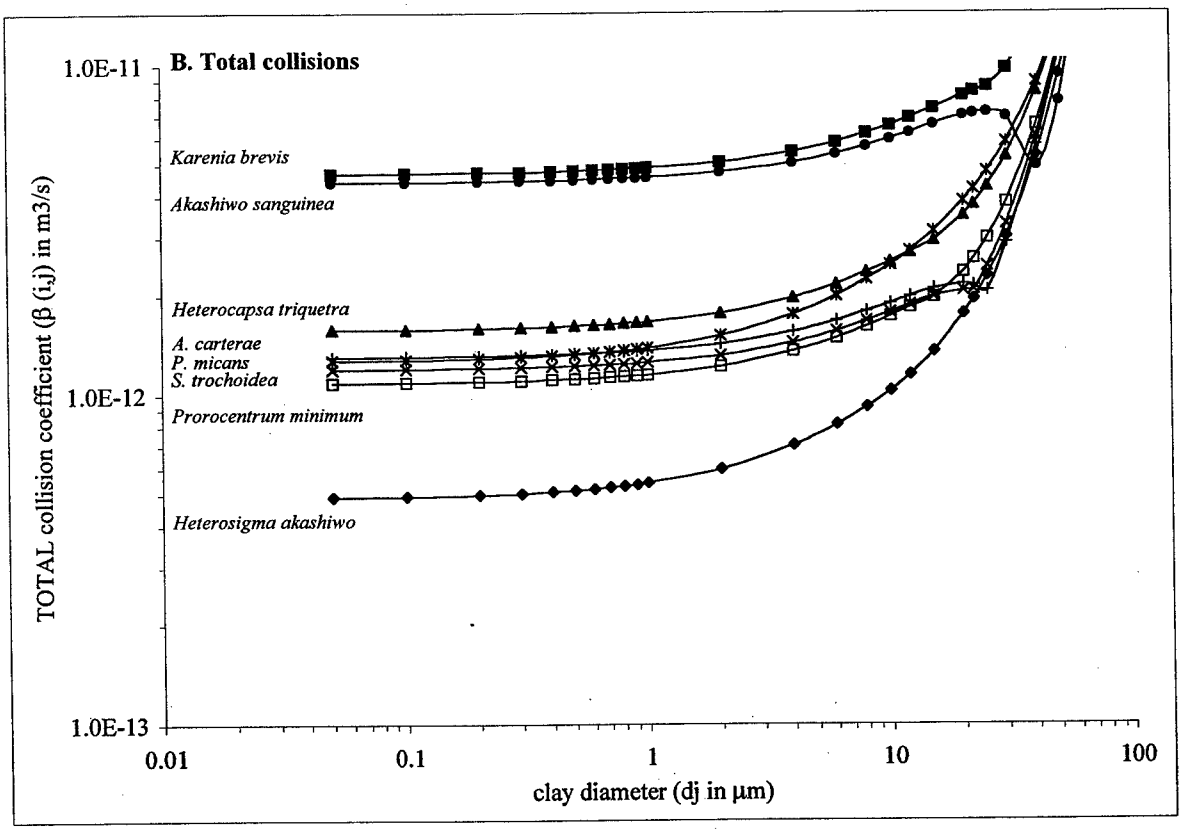
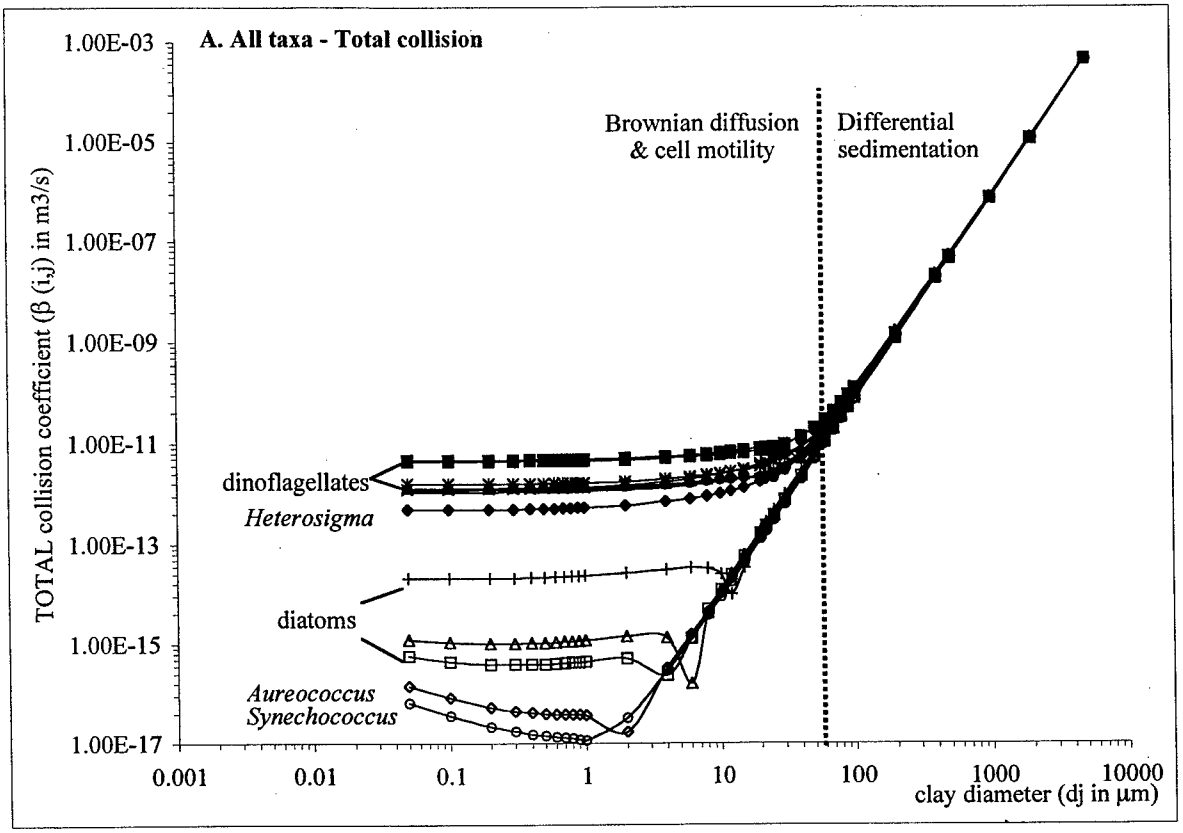
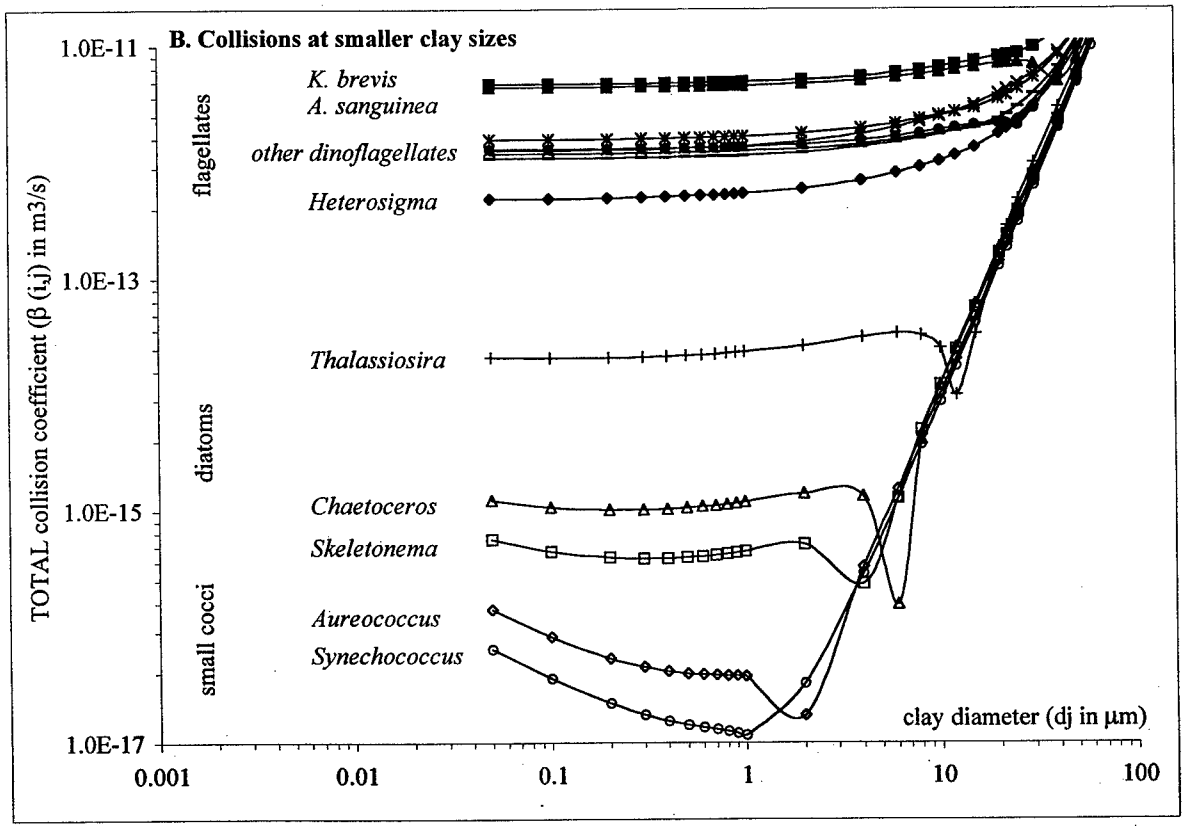

B. Total collisions
1.0E-11
1.0E-12
1.0E-13
TOTAL collision coefficient (β (i,j) in m3/s)
Karenia brevis
Akashiwo sanguinea
Heterocapsa triquetra
A. carterae
P. micans
S. trochoidea
Prorocentrum minimum
Heterosigma akashiwo
0.01
0.1
1
10
100
clay diameter (dj in µm)

Figure 3-11. Comparison of collision frequency coefficients for all algal taxa in the survey as a function of clay particle size. (A) Total collisions include Brownian diffusion, swimming motility (for flagellates) and differential sedimentation. (B) comparisons in the clay size are dominated by diffusion and motility. Cell diameters used in the calculation of collision frequency coefficients are listed in Table 3-2.


A. All taxa - Total collision
Brownian diffusion & cell motility
Differential sedimentation
TOTAL collision coefficient (β (i,j) in m3/s)
1.00E-03
1.00E-05
1.00E-07
1.00E-09
1.00E-11
1.00E-13
1.00E-15
1.00E-17
dinoflagellates
Heterosigma
diatoms
Aureococcus
Synechococcus
0.001
0.01
0.1
1
10
100
1000
10000
clay diameter (dj in µm)


B. Collisions at smaller clay sizes
TOTAL collision coefficient (β (i,j) in m3/s)
1.0E-11
1.0E-13
1.0E-15
1.0E-17
flagellates
diatoms
small cocci
K. brevis
A. sanguinea
other dinoflagellates
Heterosigma
Thalassiosira
Chaetoceros
Skeletonema
Aureococcus
Synechococcus
clay diameter (dj in µm)
0.001
0.01
0.1
1
10
100

ming alone (Figure 3-8C, $R^2 = 0.90$). This suggests that cell size and motility may need to be considered together. In addition, motility as a collision mechanism for the flagellates remained important until clay particle size reached 50 μm, in this system where water flow was assumed to be minimal. For the non-motile, minute *Synechococcus* and *Aureococcus*, however, collisions are generated mostly by diffusive processes but shift to differential sedimentation as particle sizes increase during aggregation. Lastly, the diatoms, with their larger cell sizes, depend solely on differential sedimentation to produce interparticle collisions in quiescent systems.

Mixed culture experiments. In the first set, *Karenia brevis* (at 1100 cells ml^{-1}) was combined with two concentrations of the dinoflagellate *Prorocentrum micans* (430 and 1600 cells ml^{-1}) (Figure 3-12). Compared to *K. brevis* alone, the removal of *K. brevis* increased in the presence of *P. micans*, particularly when clay loading was > 0.10 g L^{-1}. It also increased with increasing *P. micans* concentration. By contrast, the RE of *P. micans* alone, at both cell concentrations, was < 20% as reported above. The removal values increased slightly in the presence of *K. brevis*, but only up to 50% with 0.50 g L^{-1} of clay (Fig. 3-12B).

In the second set of experiments, *K. brevis* (at 2100 cells ml^{-1}) was combined with two concentra-tions of the diatom *Skeletonema costatum* (35,000 and 300,000 cells ml^{-1}) (Figure 3-13). Again, the removal of *K. brevis* in the presence of the diatom increased compared to *K. brevis* alone, and the effect was most pronounced when *S. costatum* concentration was high and the clay loading was at 0.10 g L^{-1}. The RE of *S. costatum* was very low, even with *K. brevis* present. However, the variability was large, making it difficult to assess whether the removal of *S. costatum* was enhanced when *K. brevis* was present (Fig. 3-13B).

Mesocosm experiments. The initial concentration of *Karenia brevis* during a bloom in Texas coastal waters was 168 cells ml^{-1} (SD = 4.86, n = 4). Three of the most abundant co-occurring species were *Prymnesium* sp. (213 cells ml^{-1}, SD = 16.9) and the two diatoms *Skeletonema* sp. (107 cells ml^{-1}, SD = 26) and *Bacillaria* sp. (151 cells ml^{-1}, SD = 9.03). The minor constituents of the community were all less than 70 cells ml^{-1} and were not considered in this study: *Prorocentrum* spp., *Chaetoceros* sp., *Pseudonitzschia* sp., *Thalassiosira* spp., *Thalassionema* sp., *Cylindrotheca* sp. and *Rhizosolenia* sp. There

was a loss of 20.9% of *Karenia* sp. from the control tanks without clay treatment, and a RE of 58.2% following 0.05 g L^{-1} clay treatment. After factoring out the loss from the untreated control, the removal dropped to 48.9% (Fig. 3-14). From laboratory trials, the expected removal efficiency of *Karenia brevis* alone at this concentration (< 200 cells ml^{-1}) should have been less than 10%. Therefore, the removal rate from the mesocosm was almost five times higher than expected.

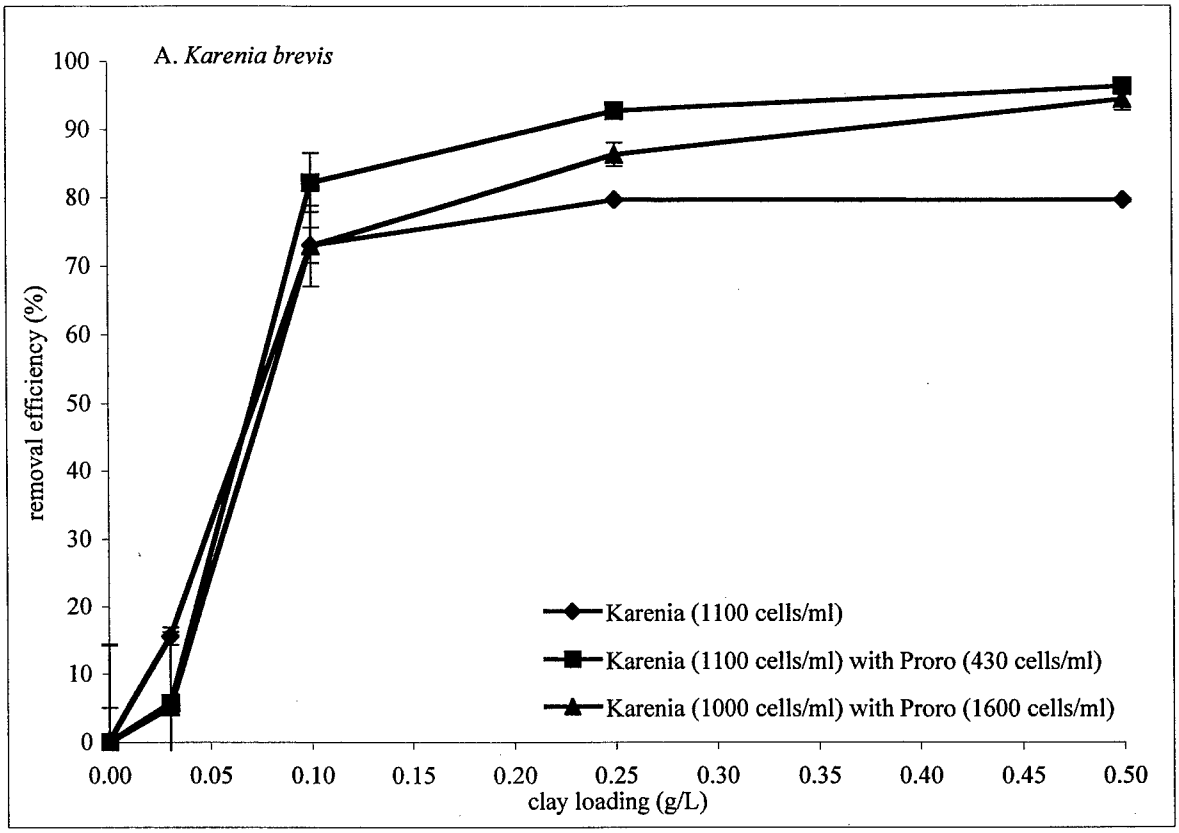
There was also sinking loss in the control tank for *Bacillaria* sp. and a slight loss for *Prymnesium* sp. On the other hand, *Skeletonema* sp. was stabilized during the 2.5 hour clay treatment, perhaps due to cell growth in the tank. After factoring out the controls, the removal efficiency of *Bacillaria* sp and *Skeletonema* sp. were 34.0% and 55.1%, respectively (Fig. 3-14). Finally, the change in *Prymnesium* sp. concentration was very slight, but the variability in the counts was high. Based on a one-way ANOVA analysis, there is no statistically significant differences among the various removal efficiencies from the four species ($P = 0.167$).

Discussion

A number of studies have demonstrated that fine dispersions of clay minerals can remove algal cells from seawater (Avnimelech et al., 1982; Soballe and Threlkeld, 1988; Yu et al., 1994b; Sengco et al., 2001). Moreover, several of these studies have shown that some species are removed more efficiently than others, but the mechanism underlying this differential removal is not clearly understood. In this study, we present laboratory and mesocosm data that demonstrate differential removal of algal species in both single, unialgal cultures and in natural mixed assemblages. To elucidate the mechanisms involved, the effect of algal size and motility were studied to predict how they may influence the frequency of collisions between clays and cells, according to physico-chemical aggregation theories. Lastly, the effect of clay and cell concentrations were also tested to determine how these would change the overall empirical removal rates.

Removal efficiency and concentration. In almost all cases, the removal efficiency of the organisms increased with clay loading. The same finding has been made in previous studies using montmorillonite (e.g. Shirota, 1989, Na et al., 1996). At low clay dosage, however, removal efficiency shifted to negative values indicating a stable sus-

Figure 3-12. Removal efficiency of *Karenia brevis* and *Prorocentrum micans* in mixed cultures, treated with IMC-P2 phosphatic clay. (A) Removal efficiency of *K. brevis* alone (1100 cells ml^{-1}) and mixed with two concentrations of *P. micans* at 430 cells ml^{-1} and 1600 cells ml. (B) Removal efficiency of *P. micans* alone, at 500 cells ml^{-1} and 1600 cells ml^{-1}, and mixed with *K. brevis* at 1100 cells ml^{-1}.


A. Karenia brevis
removal efficiency (%)
clay loading (g/L)
Karenia (1100 cells/ml)
Karenia (1100 cells/ml) with Proro (430 cells/ml)
Karenia (1000 cells/ml) with Proro (1600 cells/ml)

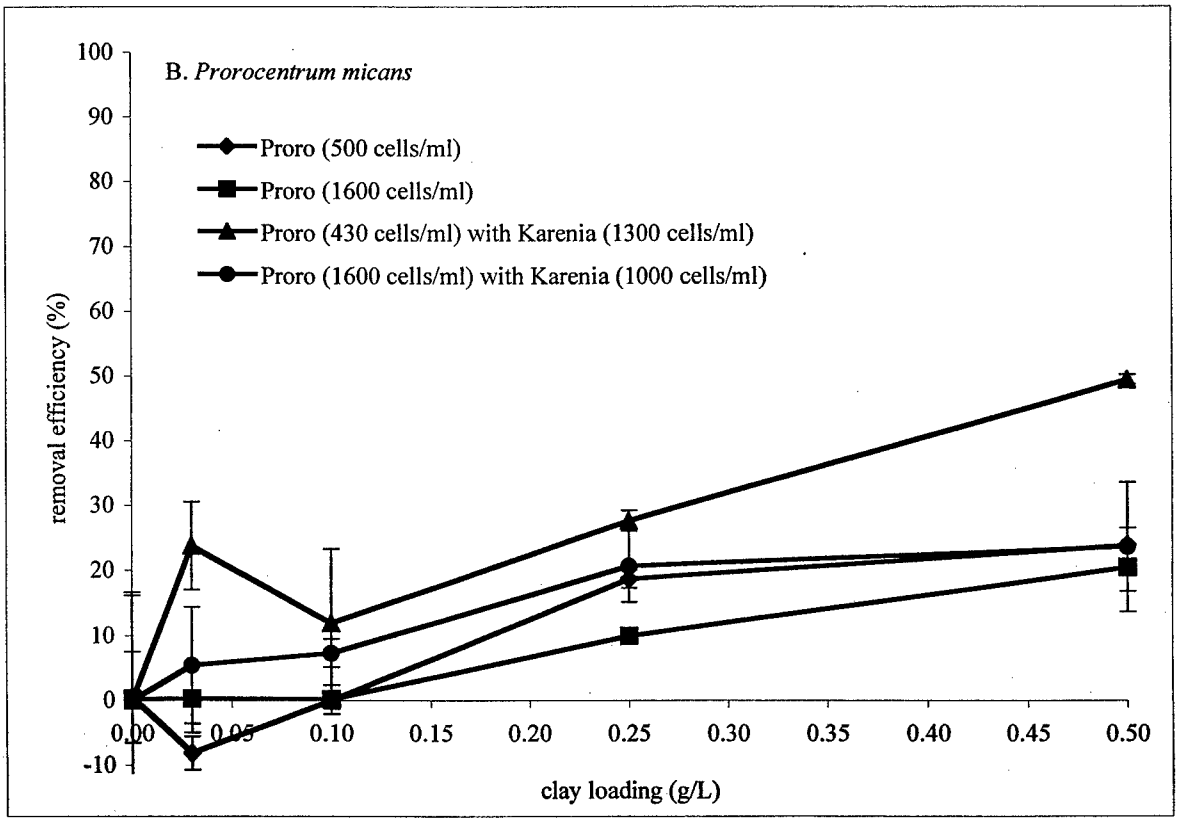
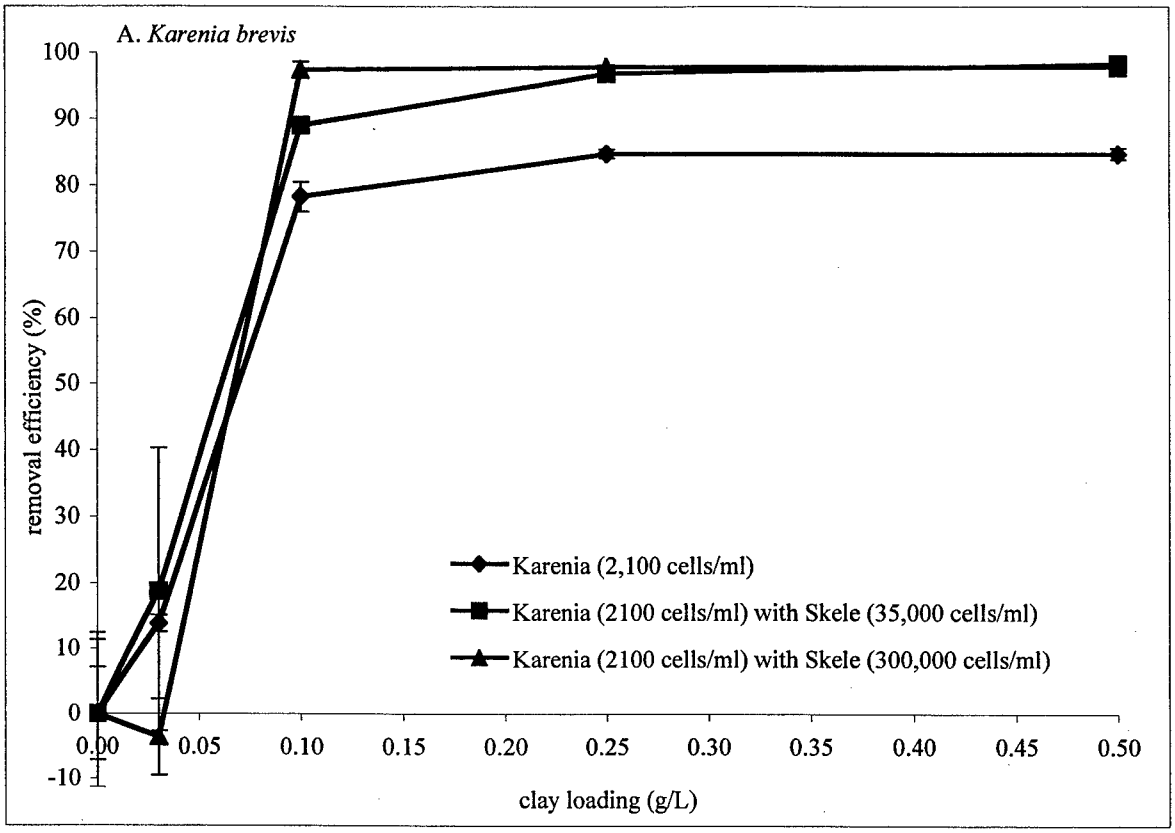

B. Prorocentrum micans
removal efficiency (%)
clay loading (g/L)
Proro (500 cells/ml)
Proro (1600 cells/ml)
Proro (430 cells/ml) with Karenia (1300 cells/ml)
Proro (1600 cells/ml) with Karenia (1000 cells/ml)

Figure 3-13. Removal efficieny of *Karenia brevis* and *Skeletonema costatum* in mixed cultures, treated with IMC-P2 phosphatic clay. (A) Removal efficiency of *K. brevis* alone (at 2100 cells ml^{-1}) and mixed with two concentrations of *S. costatum* at 35,000 cells ml^{-1} and 300,000 cells ml^{-1}. (B) Removal efficiency of *S. costatum* alone, at 35,000 and 300,000 cells ml^{-1}, and mixed with *K. brevis* at 2100 cells ml^{-1}.


A. Karenia brevis
removal efficiency (%)
clay loading (g/L)
Karenia (2,100 cells/ml)
Karenia (2100 cells/ml) with Skele (35,000 cells/ml)
Karenia (2100 cells/ml) with Skele (300,000 cells/ml)

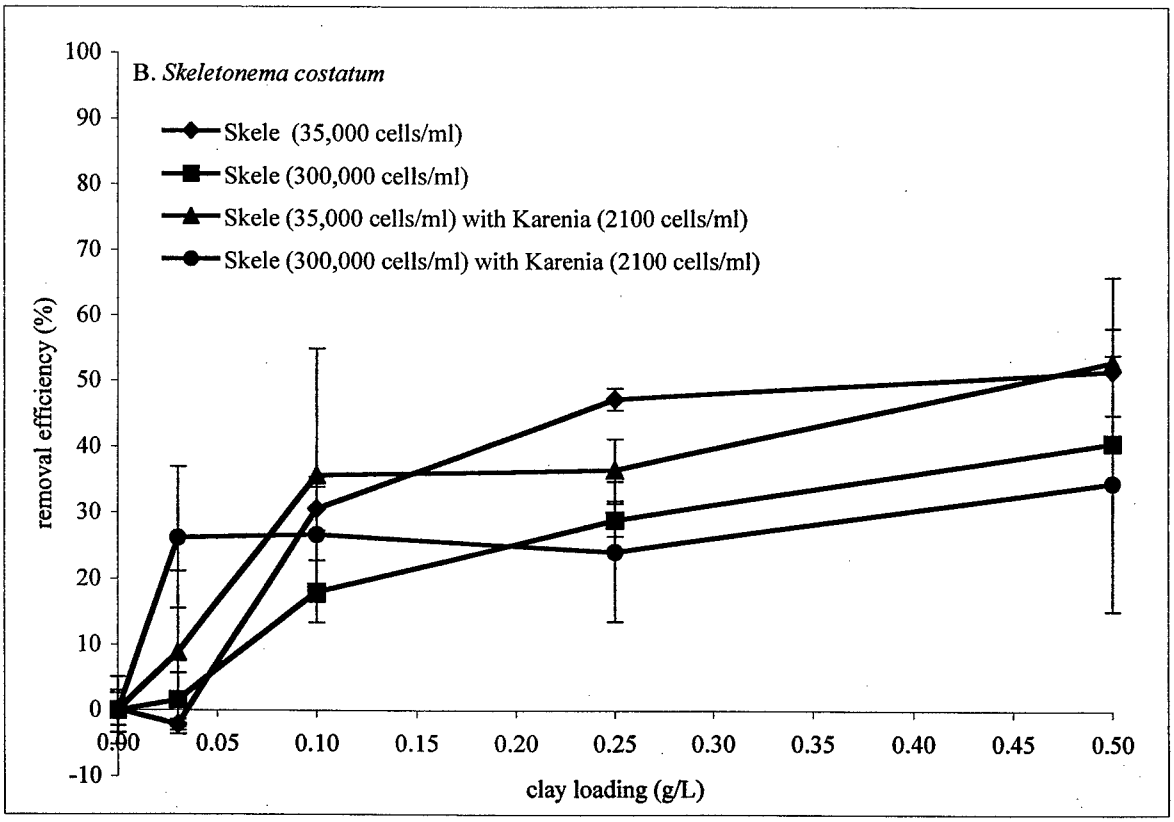
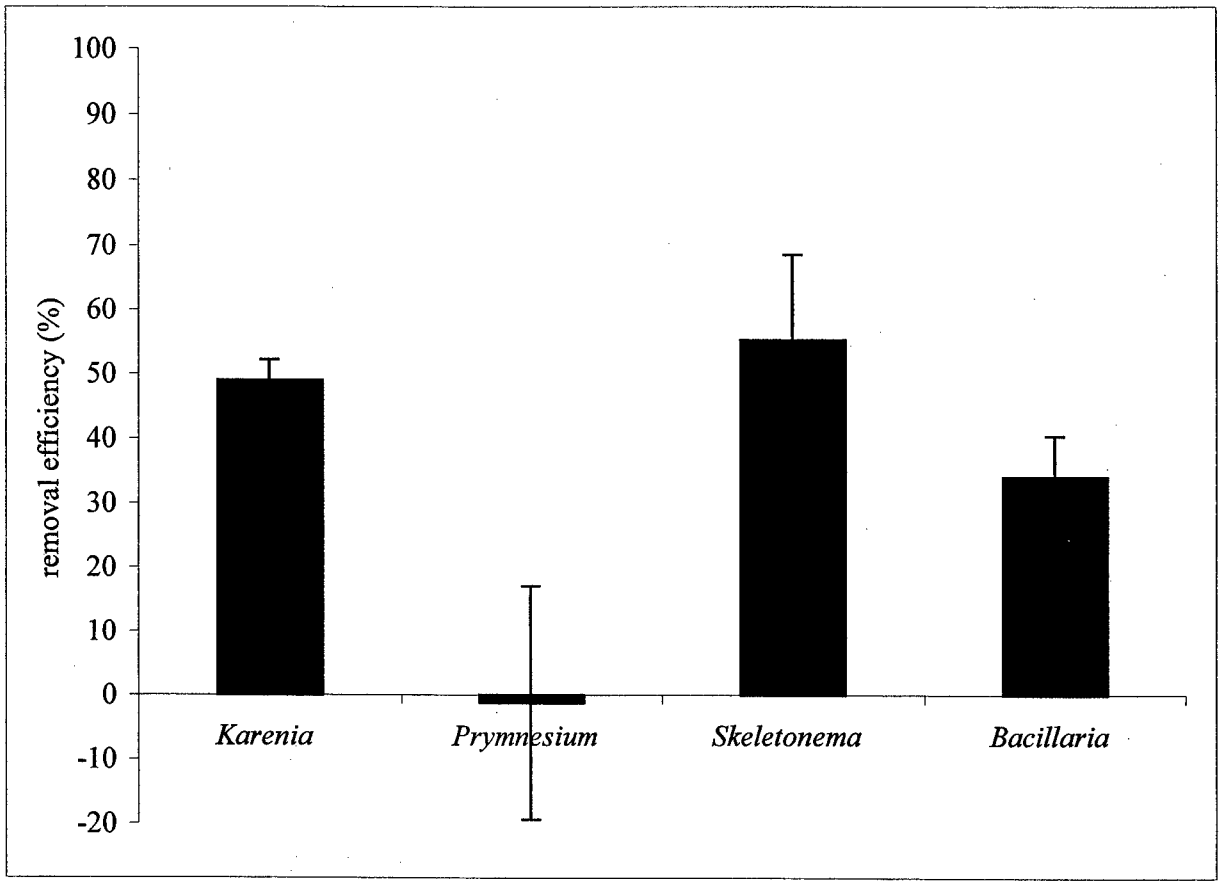

B. Skeletonema costatum
removal efficiency (%)
clay loading (g/L)
Skele (35,000 cells/ml)
Skele (300,000 cells/ml)
Skele (35,000 cells/ml) with Karenia (2100 cells/ml)
Skele (300,000 cells/ml) with Karenia (2100 cells/ml)

Figure 3-14. Removal efficiencies using IMC-P2 phosphatic clay against a natural bloom of *Karenia brevis* and a co-occuring phytoplankton assemblage in a tank mesocosm (Corpus Christi, Texas). Clay loading was 0.05 g L^{-1} and incubation time for aggregation was 2.5 hours. Error bars represent tandard deviation


100
90
80
70
60
50
40
30
20
10
0
-10
-20
removal efficiency (%)
Karenia
Prymnesium
Skeletonema
Bacillaria

pension (i.e. non-aggregating particles) which may trap cells near the surface and prevent them from sinking. In this case, the clay particle concentration may be too low to promote rapid aggregation either with itself (at the surface) or with the organisms they encounter. The aggregation rate can be described by a second order equation (O'Melia and Tiller, 1993):

$$\frac{dn}{dt} = -k_a n^2 \qquad \text{(Eq. 3-2)}$$

where n is the number concentration of particles in suspension at time t, and k_a is a rate constant that considers the physicochemical properties of the system. In this study, k_a was equated to the collision frequency coefficient β, and all collisions were assumed to produce attachment. According to this equation, the change in particle concentration with time is related to the square of the concentration of particles in the system. Therefore, increasing particle concentration can have a potentially strong effect in promoting the aggregation rate.

However, the removal efficiency did not necessarily increase when the cell concentration increased. In more than half of the species, the removal efficiency increased with increasing cell number, but for the remaining organisms, removal either increased then decreased, decreased steadily, or remained constant. These patterns may be related to the concept of an "ideal ratio" between cell numbers and clay loading for maximum removal (Avnimelech et al., 1982). The trends in the data may reflect how far the particle ratios were from this ideal value. In another study, Soballe and Threlkeld (1988) found that there were fewer effects of species concentration in the removal efficiency compared to the change in mineral concentration and differences among the species themselves. Based on empirical results, they described two modes of aggregation in their system. In the first mode, the suspension was dominated by clay particles and aggregates of clay particles (i.e. clay to clay aggregation). These particles were more numerous than the algal cells and clay-algae aggregates. They suggested that the kinetics of floc formation and number of associations between the clay aggregates and the cells would depend more on the mineral concentration than on the spacing between the algal cells or colonies. The second mode was specific to the species *Anabaena* sp. at low

mineral concentrations, one of the three organisms studied. In this case, several algal filaments would attach to a single clay mineral aggregate to form a floc (i.e. algae to clay aggregation). The authors predicted that cell concentration would be more important in this mode.

In almost all cases in this present study, low mineral (0.03 g l^{-1}) and cell concentrations simultaneously yielded negative removal efficiency, which was interpreted as a "stabilization" of the clay-cell suspension. In one extreme case, *Gymnodinium instriatum* showed negative removal even when clay dosage increased to 0.25 g L^{-1} and cell concentration increased from 500 to almost 4000 cells ml^{-1}. This observation suggested that particle concentration is not an important factor in this particular system. Particle stabilization, even at the high ionic strength of seawater, may be affected by steric effects due to the presence and adsorption of organic matter in the medium (O'Melia and Tiller, 1993). Burkholder et al. (1992) described a related species of *Gymnodinium* sp. that releases copious amounts of mucous-like organic matter in the presence of high silt and clay. It was considered a defense mechanism to avoid direct contact with the minerals since it later escaped from the organic sheath following sedimentation.

Removal efficiency and algal characteristics. The nine species that displayed increasing removal trends with increasing cell and clay concentrations exhibited a wide range of sizes, shapes, motility, swimming speed and taxonomic affiliations. In general, there were no apparent correlations between these factors and the efficiency of removal with phosphatic clay. While the highest removal efficiency was for two athecate dinoflagellates, there were no other distinguishable patterns between the clay's effectiveness and the type of outer structures of the cell (i.e. theca, silica frustule, cell wall, cell membrane).

Algal size (i.e. length, breadth and thickness) and the projected cross-sectional area for contact with clay also did not have a direct relationship with removal (Fig. 3-8A and B), contrary to the proposal of Yu et al. (1994b) and Sengco et al. (2001). It is important to note that the diatom species used by Yu and his colleagues had much larger dimensions than those used in this study. Moreover, the clays were initially mixed thoroughly with the cells which may affect the contact between particles. In these experiments, the clay was added at the surface of the tubes and allowed to settle from there.

Removal efficiency and the collision frequency coefficient. In Eq. 3-2, the aggregation ratedepends on k_a which accounts for the physical and chemical properties of the system. Focusing on transport processes and particle collisions, there was better agreement between the calculated total collision frequency coefficients and the removal patterns for the species. For the flagellates, collisions due to cell motility contributed significantly to the calculation, especially when clay sizes were relatively small (< 50 μm). Furthermore, this calculation considers both cell size and swimming speed. Considering *Karenia brevis* and *Akashiwo sanguinea*, the two species with the highest removal efficiencies, *K. brevis* is smaller than *A. sanguinea*, but it swims faster. Therefore, the two factors were balanced and the two species were removed comparably by clays. For the non-motile diatoms and small cocci, the most important transport mechanism is differential sedimentation when clay particle size exceeds 1 μm. However, the effectiveness of this mechanism is reduced further, theoretically, when the difference between the sizes of the two particles becomes too great (McCave, 1984) due to hydrodynamic retardation (O'Melia and Tiller, 1993; Thomas et al., 1999). Therefore, the smallest species may be relatively unaffected by the clay, across a range of dosages and cell concentrations. Indeed, our smallest species (e.g. *Synechococcus* WH8017 and *Aureococcus anophagefferens*) had RE's of 25% or less.

Due to the quiescent design of these experiments, collisions due to water motion were likely very small, and thus were not considered. While the effect of water motion on this system was not included, some theoretical calculations suggested cell motility would remain dominant for flagellates even when shear rates are close to 30 s^{-1} (Appendix A-5). However, velocity gradients are important in promoting collisions for the smaller cells, and for breaking up the clay flocs to reduce hydrodynamic effects (Sengco et al., 2001). In fact, the removal efficiency of *Synechococcus* WH8017 reached 62% removal when clay (at 0.25 g/L) was mixed thoroughly into the medium following addition, rather than simply layering it on the surface (Appendix A-6).

According to the analysis of collision freqeuncy coefficients, differential sedimentation becomes an important mechanism for promoting particle collisions in the clay size range > 50 μm (Fig. 3-9 and Fig. 3-11A). In theoretical calculations (Table 3-1), the relative density of the particle, ρ, is a key factor in process and can affect the outcome of

this collision mechanism. For the present study, this factor was constant and given the value of clay density (i.e. ρ_{clay} = 2.64 g cm^{-3} for phosphatic clay). However, this estimate may be larger than the actual density of the clay-cell due to the porosity of the agglomerates and the incorporation of seawater in the flocs. Since the clay-cell aggregates tended to sink, it was clear that the floc density was greater than that of the medium and that this value may serve as the lower value. Therefore, the true density of the aggregate would lie in between 2.64 g cm^{-3} (clay density) and 1.02 g cm^{-3} (seawater density at 25 C, salinity = 29.6). It is unclear, however, how to obtain the actual density of the particles for the calculation of differential sedimentation, given that they form in a dynamic process, growing larger with time as they aggregate cell particles and cells with different densitites. Nevertheless, the contribution of differential sedimentation was calculated for 25%, 50% and 90% of the change in aggregate density (Appendix A-7). This analytical treatment suggested that the relative importance of cell motility and differential sedimentation remain the same (i.e. motility is more important for the smaller range of clay particle size relative to differential sedimentation. However, the point at which the transition from cell motility to differential sedimentation moved towards the right (i.e. at higher clay particle sizes, from 50 to 105 μm when the floc was assumed to be 90% seawater with a density of 162 kg m^{-3}), indicating that the influence of differential sedimentation diminishes slightly should the aggregate density decreases as the aggregate diameter increases.

Finally, the effect of particle surface properties was not addressed in this study but should be considered. Shirota (1989) attributed the effectiveness of montmorillonite clays to the higher ion-exchange and adsorptive capacity of their three-layered structure compared to the two-layered structure of kaolinites. Likewise, Yu et al. (1994a) calculated that the surface charge and the magnitude of the repulsive forces on the montmorillonite particle would have a higher degree of co-aggregation with the algal surface near the pH of seawater. In the present study, a single clay sample was used (IMC-P2 phosphatic clay). It was assumed that the stability of the clay would be very low given the high ionic strength of seawater which should effectively neutralize the particles (Stumm and Morgan, 1996). However, it is possible that the presence of

organic matter generated by the organisms themselves in culture can affect the stability of the clay by surface adsorption.

Leslie et al. (1984), Avnimelech et al.(1982) and Yu et al. (1994b) suggested that the differences in removal efficiency of organisms with montmorillonite were linked to differences in the quality and quantity of organic excretions on the cell surface. Direct observations with scanning electron microscopy revealed copious amounts of organic material surrounding *Anabaena* sp. onto which clay particles were strongly associated (Avnimelech et al., 1982). Therefore, species that exude large quantities of organic matter such as diatoms or cyanobacteria such as *Anabaena* (Hellebust, 1969) would be expected to have greater affinity with clays. Furthermore, empirical measurements of the collision efficiency (i.e. the ratio between the rate of cell to cell attachment to the rate of cell to cell collisions), or stickiness factor, for various algal species revealed that diatoms typically have higher stickiness compared to dinoflagellates (Kiorboe et al., 1990). In addition the amount of organic matter can change according to the physiological condition of the cells. The release of organic matter is lower during the early phase of the culture when nutrients are replete. More organic matter is exuded when the cells become stressed as nutrient concentration decreases. Jackson and Lochmann (1993) found low stickiness for dinoflagellates which they proposed was a mechanism for allowing these organisms to attain high bloom densities without aggregating.

Mixed species experiments and mesocosm study. When *Karenia brevis* was mixed with the dinoflagellate *Prorocentrum micans* and with the diatom *Skeletonema costatum* in cultures, the removal efficiency of *K. brevis* remained high at >75 % (at > 0.10 g L^{-1} of clay), and the values increased compared to *K. brevis* alone (Figure 3-12A and Figure 3-13A). From the previous experiment (Fig. 3-1A), the increase in removal can be attributed to the increase in the total number of cells in the combined cultures.

With *P. micans*, its initially-poor removal gradually increased in the presence of *K. brevis* relative to *P. micans* alone. The same results was found with *S. costatum*, although there was a high variability in the cell counts. The removal of both species were much lower than *K. brevis*.

In field mesocosm experiments, the same enhancement of removal was observed for *Karenia* sp. in the presence of other species. The removal of *Karenia* sp. was higher

than expected from unialgal laboratory trials, by a factor of five. The removal of *Skeletonema* was also higher than expected given the initial concentration in the field and the loading rate of clay. There are two possible explanations for these enhancements: (1) *Skeletonema* sp. in the field existed as long chains of larger cells compared to the solitary and smaller cells in laboratory cultures. (2) The seawater appeared rich with organic matter based on its color and the large amount of foam on the surface of the tanks, and the slippery, viscous texture of the water. Organic matter may enhance cell removal by acting as bridging polymers between cells and clay particles. Finally, the removal of *Prymnesium*, a small flagellate, was very small during the treatment, suggesting that clay treatment may not affect this species.

These mesocosm results suggest that the removal efficiency of *Karenia* sp. with IMC-P clay remains feasible under field conditions, even at relatively low cell concentrations . Despite laboratory results indicating that removal will be low at low cell concentrations (present study), *Karenia* sp. may still be removed effectively in a field setting, possibly due to the presence of other species and organic matter to enhance stickiness. Moreover, the lower removal of other, non-target species relative to *Karenia* sp. is a good result in the context of environmental impacts since a clay treatment would not be expected to create a "biological desert". However, this has been a single study focusing on a small component of the total planktonic community. Additional studies are needed (1) to focus on short-term removal and impacts, as well as (2) long term incubations to determine the recovery of the community, and the possible changes in the composition and abundance of individual or groups of species long after the clays have settled.

Implication for phytoplankton community. In theory, the ability of clay minerals to remove algal species differentially may have ecological consequences in natural systems. Based on this study, one possible *direct* impact of clay dispersal on the phytoplankton community is a change in the species composition of the community. For example, the mutual aggregation and sedimentation of certain algae with clays can lead to the preferential removal of co-flocculating species to the sediment, while the resistant species may become enriched at the surface (Avnimelech et al., 1982). Hence, the species composition and relative abundance of groups will be affected (Soballe and Threlkeld, 1988). While there have been numerous studies of clay removal using

individual species, there have been no previous reports of clay treatment of water masses containing a mixture of algal species.

Community effects occur over both short and long time scales. The removal process with clay begins within hours of addition. Within this time frame, the abundance and composition of the community will be directly affected. However, cell growth in the remaining community can begin to replace the eliminated organisms. Also, cells can escape from the flocs and grow, depending on the clays dosage, depth, and the hydrodynamics of the water column (Sengco et al., 2001). In the long term, clay treatment may affect the successional patterns in the community.

Clay dispersal may also have *indirect* impacts on the phytoplanktonic community. For example, Lind et al. (1997) studied the dynamics of phytoplankton and bacterioplankton populations in turbid (clay) suspensions in freshwater limnocorral experiments. Light attenuation limited phytoplankton production and this reduction in primary production negatively affected bacterioplankton production. However, the growth of bacterioplankton remained high and was mediated through the consumption of concentrated organic matter adsorbed on the clay particles. In another limnocorral study, Cuker (1987) observed a reduction in net community productivity (NCP), chlorophyll *a* concentration and algal density with the addition of 100 g m^{-2} d^{-1} of kaolinite. The community also shifted from the dominant blue-green *Spirulina major* to *Trachelomonas superba* and other flagellates. The opposite effect on productivity was found with P enrichment (3.3 mg m^{-2} d^{-1}), with increases in diatom and chlorophyte numbers and dominance by two nitrogen-fixing *Anabaena* species. The combination of kaolinite and phosphorus addition produced intermediate values of NCP and chlorophyll *a*, and it appeared that clay addition eliminated the effect of P fertilization on the algal community, yielding mostly flagellates and a decrease in diatom concentrations. Clay superceeded phosphorus enrichment in organizing community structure.

Additional studies will be need to address the questions of long term and indirect impacts due to clay addition and the differential removal of algal species. Information from such work will be critical in understanding the feasibility and practicability of this method in controlling harmful algal blooms.

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CHAPTER 4

The Electrophoretic Mobility and Zeta Potential of Marine Microalgae and Clay Mineral Particles Suspended in Natural Seawater

Abstract

The electrophoretic mobilities (EPM) of nine marine microalgal species, representing three taxonomic classes (i.e. Bacillariophyceae, Chrysophyceae and Dinophyceae), were measured. The motility of the dinoflagellates was arrested by vortexing small aliquots of cell cultures from 30 s to 2 min, depending on the species, sufficient enough to stress the cells without killing them. The cessation of motility and the eventual recovery were monitored using light microscopy. All of the species displayed a slight electronegative charge as measured by EPM, ranging from -0.19 to -0.57 $x10^{-8}$ m^2 s^{-1} V^{-1}. The corresponding zeta potential (ζ) ranged from -2.5 to -7.6 mV. These values predict an unstable suspension, although cell-cell aggregation was not observed possibly due to steric stabilization. These values confirmed the prediction that marine algal cells exhibit negative charge similar to their freshwater counterparts, although the magnitude of the charge and ζ is lower than those in related freshwater species. These data represent the first such measurements from marine species and for flagellated species. However, there were no significant differences in the ζ to explain the propensity of certain species (e.g. *Gymnodinium breve* and *Heterocapsa triquetra*) to be removed preferentially over others by a given clay mineral, as described in the previous reports.

The EPM of twelve clay minerals, including phosphatic clays, montmorillonites, kaolinites and zeolites, were determined in both freshwater (distilled/deionized) and natural seawater from Vineyard Sound, MA (salinity = 29.6, pH = 8.35). All of the mineral types displayed a negative charge in the freshwater medium, except for one kaolinite (i.e. H-DP, EPM of 3.9 $x10^{-8}$ m^2 s^{-1} V^{-1}) which had been treated with a cationic polymer by the manufacturer. All of the bentonites and one zeolite (SW-NM) showed the highest EPM (> -3.0 $x10^{-8}$ m^2 s^{-1} V^{-1}) and ζ (> -37 mV). Hence, the bentonites, SW-NM zeolite and H-DP kaolinite were determined to be the most stable in freshwater. The remaining kaolinites and zeolites displayed a range of values. All of the phosphatic clays were among the least negatively-charged samples (EPM of -1.6 to -1.7 $x10^{-8}$ m^2 s^{-1} V^{-1}), and their ζ (-21.9 to -20.1 mV) indicated a relatively unstable suspension in freshwater. In natural seawater, the EPM and ζ for all of the clays were reduced to a more uniform, slightly electronegative value. In addition, rapid aggregation and settling were observed in all clay preparations in this medium. These results were consistent with

the expectation that clay minerals possess a negative surface charge in freshwater, and that this charge is reduced as the ionic strength of the medium increases. Moreover, uniformity in the EPM agreed with previous determinations of inorganic particles in natural seawater which is thought to be caused by the adsorption of surface-active organic substances. To determine the change in ζ with increasing salinity, four selected clays were suspended in media with increasing salinity. At the highest dilution (salinity = 1.9), all of the clay samples were already negatively charged, including the polymer treated kaolinite H-DP. At salinity = 3.7 and higher, the values varied slightly with increasing salinity (ζ = -12 to -20 mV). While there were marked differences between the stability of the suspensions in fresh-water, these differences disappeared quickly as the salinity increased with the largest change occurring with the bentonite WB-B and the polymer treated kaolinite H-DP. The information from ζ was insufficient to immediately explain the effectiveness of one mineral type over another in removing a given algal species.

Introduction

For over a decade clay minerals have been investigated and applied as a way of directly controlling the spread and impacts of harmful algal blooms (HABs), an aquatic phenomenon caused by the growth and accumulation of certain microalgal species (e.g. Maruyama et al., 1987; Yu et al., 1994b, 1995; Na et al., 1996; Sengco et al., 2001). The principle behind this strategy is the mutual aggregation between the organisms and the clay particles, leading to the formation of large flocs which settle to the ocean floor. In the process, the cells are removed physically from the water column and are sometimes killed by surface contact with clay (Shirota, 1989; Bae et al., 1998; Sengco et al., 2001). The reduction in bloom density near the surface by clay dispersal has effectively mitigated the potentially deleterious impacts of the HAB in the areas of extensive mariculture production in Japan (Shirota, 1989) and South Korea (Choi et al., 1998). In these trials, the removal efficiency of minerals such as montmorillonite and yellow loess clay (i.e. a mixture of gibbsite, quartz and kaolinite) reached over 90% with no reported mortality in the cultured fish due to clay treatment, and it resulted in the improvement of water transparency and the recovery of moribund fish. As these and other research programs continue to develop in order to understand the effectiveness and possible impacts of clays, clay dispersal has emerged as one of the most promising and practicable control strategies for HABs (Anderson, 1997).

The chief process involved in the removal of HABs with clay is particle aggregation. Typically, aggregation has been divided into two sequential steps (O'Melia and Tiller, 1993): particle transport and attachment. Transport brings the particles together and it is a physical process governed by hydrodynamics and external forces such as gravity. The dominant mechanisms include molecular or Brownian diffusion, fluid motion (laminar and turbulent), and differential sedimentation. In the clay-cell system, a significant number of particle contacts may also be generated by the motility of flagellated species such as dinoflagellates (Jackson and Lochmann, 1993; Chapter 3). In the second step of aggregation, particle attachment is governed mostly by the surface chemical properties of the particles and the chemistry of the surrounding medium (e.g. pH, ionic strength). For clay minerals, surface charge develops from (1) isomorphic substitutions (i.e. the exchange of ions with lower valences in the crystal structure), (2)

surface reactions on the clay surface (e.g. H^+, OH^-), and (3) specific adsorption of charged molecules onto the surface (e.g. metals, organic molecules, surfactants, and polyelectrolytes). These charges, usually negative, are balanced by ions of opposite charge in the medium (i.e. positive counterions), thus creating the so-called double layer arrangement. When particles collide and their similarly-charged double layers interact, they are repelled by electrostatic forces and the rate of aggregation is low (i.e. a stable suspension). As the ionic strength increases, however, the double layer is compressed by the growing number of counterions allowing the ever-present but shorter-range attractive forces (e.g. London-van der Waals) to dominate, thus promoting aggregation (i.e. an unstable suspension). Clay particles may also attach by polymer bridging, a case where parts of a long-chained molecule (e.g. polyelectrolytes, flocculants) bind to two different particles. However, these molecules may also stabilize the suspension by binding extensively along the particles' surfaces and preventing contacts when parts of the molecule interact from two such coated particles (steric stabilization).

Previous measurements of surface charge on clay minerals in low ionic strength media have consistently revealed a negative charge (e.g. van Olphen, 1963; Bayne and Lawrence, 1972). As the ionic strength increases, however, the double-layer thickness decreases resulting in faster aggregation rates. In laboratory and field experiments, Edzwald et al. (1974) not only found the destabilization of mineral particles with increasing salinity but that the various minerals also displayed varying rates of destabilization: illite was more stable than kaolinite which was more stable than montmorillonite. Moreover, the amounts and distribution of these minerals in the sediment, along the increasing salinity gradient from river to upper estuary, reflected the patterns of stability: the less stable kaolinite was found where salinity was lower while, illite was found farther downstream where salinity was highest. In several studies focusing on the settling velocity of different clays with increasing salinity, montmorillo-nite was found to have the lowest rate, followed by kaolinite and illite (Whitehouse et al., 1960; Postma, 1967; Hahn and Stumm, 1970; van Leussen, 1988). Hahn and Stumm suggested that these patterns also reflected their different stabilities, although the pattern is reversed from that found by Edzwald et al. (i.e. montmorillonite is more stable than kaolinite which is more stable than illite). At ion concentrations approaching seawater, Stumm and Morgan (1996)

predicted that the thickness of the double layer would be < 1 nm, about equal to the size of a hydrated ion, and thus aggregations would occur. However, stabilization can occur through specific adsoption as demonstrated repeatedly in various studies of natural oceanic particles coated by humic matter (Neihof and Loeb, 1974; Hunter and Liss, 1979; Hunter, 1980; Hunter and Liss, 1982; Loder and Liss, 1985). Moreover, Hunter and Liss (1979) suggested that the effects of differing surface and electrical properties of minerals, which would lead to differential aggregation with increasing ionic strength, may be nullified by the adsorption of surface-active organic matter in the water which creates uniform charges in different particles.

Microalgal cells display a wide variety of surface features that can influence its surface charge and its propensity for aggregation (i.e. stickiness). For example, diatoms are surround by a silicious shell called a frustule and some members of the dinoflagellates have a theca composed of cellulose. Other species have cell walls or mucilagenous sheaths. More importantly, algal cells are also surrounded by various types of organic molecules such as nucleic acids, lipids, glycoproteins and carbohydrates (Dodge, 1973). Not only do these molecules have structural significance to the cell, but they have important roles in the functioning of cells including nutrient binding and acquisition, transport of molecules across the cell membrane(s), maintaining ionic balance, and buoyancy control. The surface charge of microalgae is thought to be generated by the ionization of these molecules (Kreger, 1962; Tenney et al., 1969; Maruyama, 1987). Ives (1956) was the first to determine the surface charge of several freshwater species using electrophoresis and found that they carried a negative charge. However, the author made no observations using flagellated species because the swimming ability of these organisms interferred with their motion in the electric field, so there have been no studies on flagellated microalgae to date. Geissler (1958) also reported a negative charge on several freshwater diatoms and confirmed the finding by observing the strong attachment of positively-charged dye particles onto the surface. Tenney et al. (1969) demonstrated the binding of cationic polymers on the cell surface and postulated that the association with the cell surface was electrostatic instead of chemical in nature. While several authors have speculated that the surface charge of marine microalgae is also negative (e.g. Yu et al., 1994a), there have been no direct measurements made.

The first goal of this paper was to determine the surface charge and electrokinetic (zeta) potential of several marine microalgal species, including dinoflagellates, which have been used previously in removal experiments with clay. The hypothesis was that the removal efficiency of different organisms with a given mineral type is related to differences in the surface charge and zeta potential of each organism. These measurements may also add information on how charge and potential may interact with cell size, concentration and behavior (i.e. swimming speed) in differential removal with a particular clay mineral. For instance, Yu et al. (1994b) attributed the higher removal efficiency of two diatom species, *Nitzschia pungens* and *Skeletonema costatum*, to their large cell size (and thus large surface area for clay attachment) and to their high stickiness due to the mucilaginous excretions often associated with diatoms. Sengco et al. (2001) observed that the removal of *Heterosigma akashiwo* and *Gymnodinium breve* with phosphatic clay (i.e. a montmorillonite-rich deposit from phosphate extraction in Florida) was higher than for *Alexandrium tamarensis*, a much larger cell. The authors proposed that cell stickiness for the clay may be more significant in co-aggregation than algal size.

The second goal of this paper was to determine the surface charge and zeta potential of various clay minerals in both freshwater and seawater. As before, the hypothesis was that the removal ability of clay minerals for a given species is associated with the surface charge and potential of the clay when they are suspended in high ionic strength. In addition, differences in removal ability may be related to the rate at which the clays are destabilized in the water column. This is tested by taking measurements of clay suspen-sions in media with increasing salinity.

Materials and Methods

Cultures. Algal cultures were obtained from various sources (Table 4-1). They were grown in batch cultures using modified f/2+Si medium under conditions described by Anderson et al. (1999). Growth was monitored using in vivo cellular fluorescence (Model 10-AU Fluorometer, Turner Designs, Sunnyvale, California, USA) calibrated against direct microscope cell counts (Avnimelech et al., 1982). Electrophoretic mobility (EPM) measurements were performed using cultures in early to mid-exponential growth. Cell concentrations ranged from 5000-10000 cells ml^{-1}.

To arrest dinoflagellate mobility while minimizing possible changes to the surface properties, 5 ml of culture were placed in a 15-ml borosilicate test tube and vortexed between 30 s to 2 min, depending on the species. The cessation of motility was confirmed for a small aliquot placed on a depression slide and observed with a Nikon Labphot compound microscope. The recovery of motility took place for 90% of the cells within 15 min. This was sufficient time to measure electrophoretic mobility. The pH of the suspension was measured using a standard pH meter.

Clays. For freshwater suspensions, the clay samples were fractionated to obtain particles < 10 μm (Table 4-2). 0.50 g of dry clay was suspended in 1 L distilled/deionized water (DDI) with constant mixing (25°C, salinity = 0, pH = 7.41-7.83). This amount was used to minimize the hindered settling effect. For the phosphatic clays, the wet slurry (178 g L^{-1}) was diluted to the appropriate concentration with DDI water. The suspension was then placed into a glass graduated cylinder where particles >50 μm were allowed to settle for 4 minutes to a depth below 32.8 cm. The supernatant was decanted and diluted again to 1 L with DDI water. The suspension was placed into a graduated cylinder to remove particles > 10 μm by settling for 95 minutes below a depth of 31 cm. The supernatant was collected and the clay content was determined by drying several small aliquots overnight at 105°C. The electrophoretic mobility measurements were made using the fraction < 10 μm. The pH of the suspension was measured using a pH meter calibrated at pH 7.0 and 10.0 with standard buffers.

For seawater suspensions, a stock solution was prepared by dispersing 0.50 g of dry clay in 5 ml of seawater (25°C, salinity = 29.6, pH = 8.35). The seawater was collected from Vineyard Sound (MA) at the Ecosystems Laboratory (ESL) at the Woods Hole Oceanographic Institution. It was pre-filtered through a set of 1 μm cartridge filters and finally it was passed three times through a 0.20 μm cartridge filter prior to use. For the phosphatic clay, the stock slurry was diluted to the appropriate concentration using ESL seawater. The suspensions were mixed constantly and allowed to sit overnight. For the electropho-retic measurements, 0.10 g L^{-1} clay in seawater were prepared. The pH of the suspension was measured using a standard pH meter.

Electrophoretic mobility measurements. The measurements were taken on a ZetaPALS system (Brookhaven Instruments Corporation, Holtsville, NY) which utilizes

Table 4-1. Algal species used in electrophoretic mobility determination.

Clays	Mineraligy - Composition	Producer
IMC-P2	smectite mixture, carbonate-	IMC Phosphates, Inc.
IMC-P4	fluorapatite, palygorskite, mica	IMC Phosphates, Inc.
IMC-P6	kaolinite, quartz, wavelite	IMC Phosphates, Inc.
WB-B	sodium bentonite	Wyo-Ben, Inc.
SW-B	sodium bentonite	Southwest Mining Group
SP-B	Black Hills bentonite	H.C. Spinks Clay Company, Inc.
H-DP	treated kaolinite (cationic-polymer)	J.M. Huber Corporation
SP-K	kaolinite	H.C. Spinks Clay Company, Inc.
H-35	waterwash kaolinite	J.M. Huber Corporation
SE-CC	kaolinite	Southeastern Clay Company
SW-NM	Nicole mountain zeolite	Southwest Mining Group
SW-ZP	zeolite powder	Southwest Mining Group

Table 4-2. Clay samples for electrophoretic mobility determinations. Clays were suspended either in freshwater (distilled/deionized) or natural seawater (Vineyard Sound, MA).

Clays	Mineraligy - Composition	Producer
IMC-P2	smectite mixture, carbonate-	IMC Phosphates, Inc.
IMC-P4	fluorapatite, palygorskite, mica	IMC Phosphates, Inc.
IMC-P6	kaolinite, quartz, wavelite	IMC Phosphates, Inc.
WB-B	sodium bentonite	Wyo-Ben, Inc.
SW-B	sodium bentonite	Southwest Mining Group
SP-B	Black Hills bentonite	H.C. Spinks Clay Company, Inc.
H-DP	treated kaolinite (cationic-polymer)	J.M. Huber Corporation
SP-K	kaolinite	H.C. Spinks Clay Company, Inc.
H-35	waterwash kaolinite	J.M. Huber Corporation
SE-CC	kaolinite	Southeastern Clay Company
SW-NM	Nicole mountain zeolite	Southwest Mining Group
SW-ZP	zeolite powder	Southwest Mining Group

phase analysis light scattering to determine the electrophoretic mobility (EPM). The instrument was housed at the Center for Advanced Materials Processing at Clarkson University (Potsdam, NY). This device was designed to take measurements in media with high ionic strength. The autotracking feature can compensate for thermal drift during the analysis. The clay and cell suspensions were placed in plastic cuvettes and measurements were taken with 10 runs containing 50 cycles each (n = 500 measurements per sample). The analyses were conducted at 25°C. In seawater medium, the measurements were taken for 5-6 runs with 60-99 cycles each. The data represent the mean of the EPM, relative residual, zeta potential and conductance. The information was downloaded into a computer spread-sheet where the data were processed and analyzed.

Clays suspended in varying salinity. To determine the change in the electrophoretic mobility of clay minerals with increasing salinity, the ESL seawater was diluted with DDI water to yield the following salinity values: 0.0, 1.9, 3.7, 7.4, 14.8 and 29.6. The pH of the suspension was measured using a standard pH meter. 0.50 g of dry clays were suspended in the medium overnight to equilibrate. Prior to the analysis, 0.10 g L^{-1} was prepared. The same procedure above was followed.

Results

Algal cells. The protocol to arrest swimming motility was effective. Other means for stopping motility without killing the cell or affecting surface properties were not effective or feasible for this investigation. All of the species tested displayed a slight negative charge with small differences in the magnitude of the charge (Table 4-3, Figure 4-1). *Skeletonema costatum* and *Prorocentrum micans* displayed the highest EPM values with to -0.57 x 10^{-8} m^2 s^{-1} V^{-1} and a zeta potential of between -7.7 to -7.6 mV, respectively. In this paper, the units for EPM are given as 10^{-8} m^2 s^{-1} V^{-1} which is the reduced form of μm sec^{-1} cm V^{-1}. The lowest EPM and zeta potential were for the diatom *Chaetoceros simplex*. While the EPM and ζ were low for all algae, aggregation was not observed.

Clay minerals. Except for the kaolinite H-DP, all clays in this study showed a negative value in both freshwater and seawater (Table 4-4, Figure 4-2). H-DP kaolinite is a product which has been treated with cationic polymers by the manufacturer. Thus, it maintains a highly positive charge in freshwater (EPM of 3.9 x 10^{-8} m^2 s^{-1} V^{-1}, ζ = 50.2

mV). Its charge eventually shifted to negative values in seawater (EPM of -1.08 x 10^{-8} m^2 s^{-1} V^{-1}, ζ = -14.7 mV). In freshwater, all of the montmorillonites showed the highest EPM values and zeta potentials with up to -3.2 x 10^{-8} m^2 s^{-1} V^{-1} and -40.6 mV. The zeolite SW-NM had comparable values. The kaolinites and zeolites as mineral groups showed a much wider range of charges than the bentonites and phosphatic clays. Interestingly, the EPM and ζ for the various Florida phosphatic clays showed very similar values, however, the magnitudes were lower than for the pure bentonites. In freshwater, clay aggregation was not observed which agreed with the relatively high EPM (ζ) values.

In seawater, the clay mobilities and ζ were reduced and showed more uniform values. Rapid aggregation of clays in seawater was observed which agreed with the low EPM (ζ) values.

Clays in diluted seawater. The EPM of the montmorillonite WB-B decreased from -0.92 to -3.0 x 10^{-8} m^2 s^{-1} V^{-1} with increasing salinity (Table 4-5, Figure 4-3). The potential dropped from -12.5 mV to -37.8 mV with the steepest change between a salinity of 0 and 4. The polymer-treated kaolinite H-DP changed from positive to negative at ca. 1.5 salinity. The mobilities for phosphatic clay IMC-P2 and kaolinite SE-CC were relatively constant across the salinity gradient. When the salinity approached 5, the zeta potentials for all of the clays intersected between -20 mV and -22 mV (Fig. 4-3, inset). Beyond this salinity, the potentials were restricted to a band between -12 mV and -20 mV with some fluctuations among the different clay minerals.

Discussion

Algal cells. This study is the first to report on the electrophoretic mobility (EPM) and zeta potential (ζ) of marine phytoplanktonic species. It is also the first to include flagellated species which have been difficult to measure in the past because their swimming ability can readily overcome their motion in the electric field (Ives, 1956). With respect to surface charge, these data confirm the prediction that marine algal species, including the dinoflagellates, possess negative surface charges like their freshwater counterparts (Maruyama et al., 1987; Shirota, 1989; Yu et al., 1994a). However, the magnitude of the charges are smaller compared to freshwater algae. Ives (1956) reported a range of zeta potential between -7.6 mV to -11.6 mV at pH values from 7.2 to 8.8,

Table 4-3. Electrophoretic mobility and zeta potential of marine phytoplankton.

		Cells in Seawater temperature = 25 C salinity = 29.6		density = 1.0193 g/cm3 pH = 8.34-8.36 absolute viscosity = 0.946 cp	
Class	Organism	EP Mobility (10-8 m2/V·s)	Zeta Potential (mV)	Conductance (uS)	pH
Bacillariophyceae	*Skeletonema costatum*	-0.56	-7.6	81363	8.82
	Thalassiosira weisflogii	-0.22	-3.0	36280	8.95
	Chaetoceros simplex	-0.19	-2.5	28035	8.45
Chrysophyceae	*Aureococcus anophagefferens*	-0.41	-5.6	39323	8.30
Dinophyceae	*Heterocapsa triquetra*	-0.39	-5.3	49118	8.48
	Prorocentrum micans	-0.57	-7.7	49268	8.22
	Alexandrium tamarensis	-0.33	-4.5	52931	8.21
	Karenia brevis	-0.43	-5.8	57331	8.18
	Karenia mikimotoi	-0.27	-3.6	55453	8.19

Figure 4-1. Electrophoretic mobility of marine microalgae.

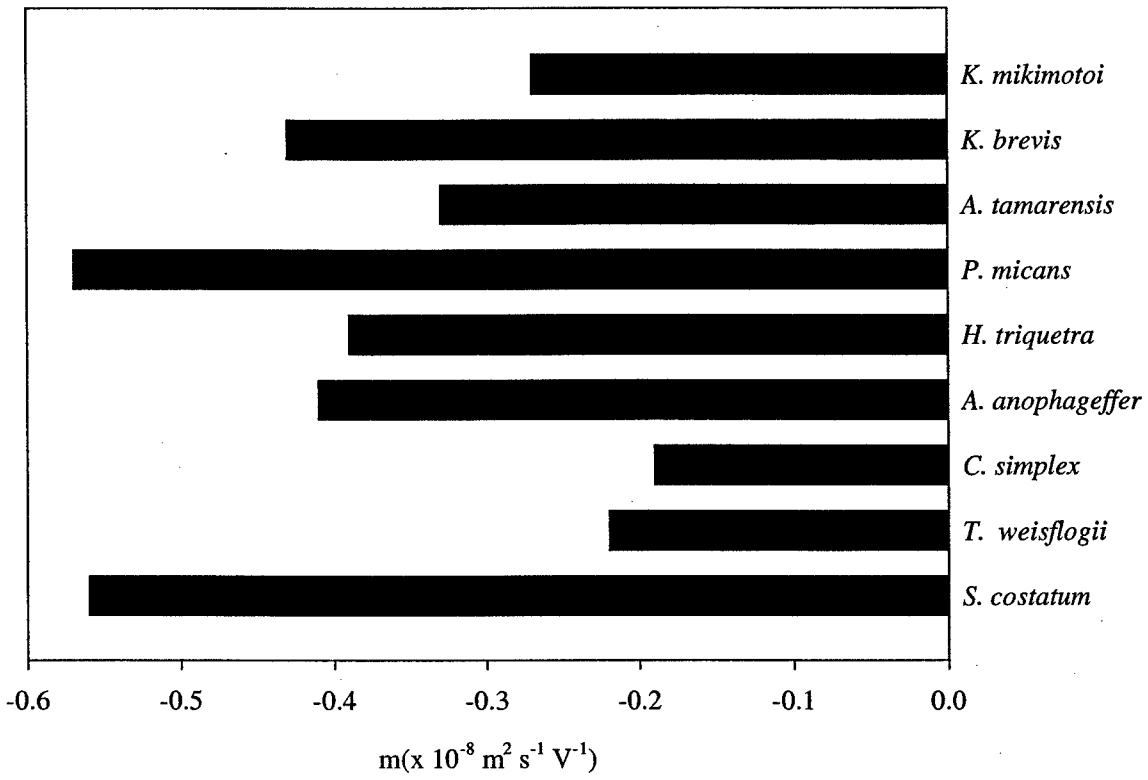

K. mikimotoi
K. brevis
A. tamarensis
P. micans
H. triquetra
A. anophageffer
C. simplex
T. weisflogii
S. costatum
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
m(x 10-8 m2 s-1 V-1)

Table 4-4. Electrophoretic mobility and zeta potential (ζ) of clay minerals in freshwater and seawater (Vineyard Sound, MA).

		Clays in Freshwater		density = 0.99707 g/cm3	
		temperature = 25 C		pH = 7.41-7.83	
		salinity = 0		absolute viscosity = 0.89302 cp	
Minerals	Clays	EP Mobility (10-8 m2/V·s)	Zeta Potential (mV)	Conductance (uS)	pH
phosphatic clay	IMC-P2	-1.6	-20.3	15	6.66
	IMC-P4	-1.6	-20.1	11	6.80
	IMC-P6	-1.7	-21.9	67	7.78
montmorillonite	WB-B	-3.0	-37.8	9	8.00
	SW-B	-3.2	-40.6	40	9.34
	SP-B	-3.1	-39.5	35	8.10
kaolinite	H-DP (with cationic polymer)	3.9	50.2	11	5.70
	SP-K	-2.1	-26.3	6	6.02
	H-35	-2.9	-37.1	25	5.99
	SE-CC	-1.6	-19.8	6	5.94
zeolite	SW-NM	-3.3	-41.8	16	6.09
	SW-ZP	-2.1	-26.5	28	6.84

		Clay in Full Seawater		density = 1.0193 g/cm3	
		temperature = 25 C		pH = 8.34-8.36	
		salinity = 29.6		absolute viscosity = 0.946 cp	
Minerals	Clays	EP Mobility (10-8 m2/V·s)	Zeta Potential (mV)	Conductance (uS)	pH
phosphatic clay	IMC-P2	-0.87	-11.9	57535	8.21
	IMC-P4	-0.81	-11.0	60292	8.27
	IMC-P6	-0.94	-12.8	58463	8.35
montmorillonite	WB-B	-0.92	-12.5	63592	8.25
	SW-B	-0.90	-12.2	58858	8.34
	SP-B	-0.76	-10.3	56036	8.34
kaolinite	H-DP (with cationic polymer)	-1.1	-14.7	68587	8.27
	SP-K	-1.0	-14.2	60452	8.33
	H-35	-0.88	-12.0	67642	8.36
	SE-CC	-1.3	-17.1	59699	8.26
zeolite	SW-NM	-0.86	-11.8	66967	8.36
	SW-ZP	-1.2	-16.3	71266	8.34

Figure 4-2. Electrophoretic mobility of clays in freshwater and natural seawater (Vineyard Sound, MA).

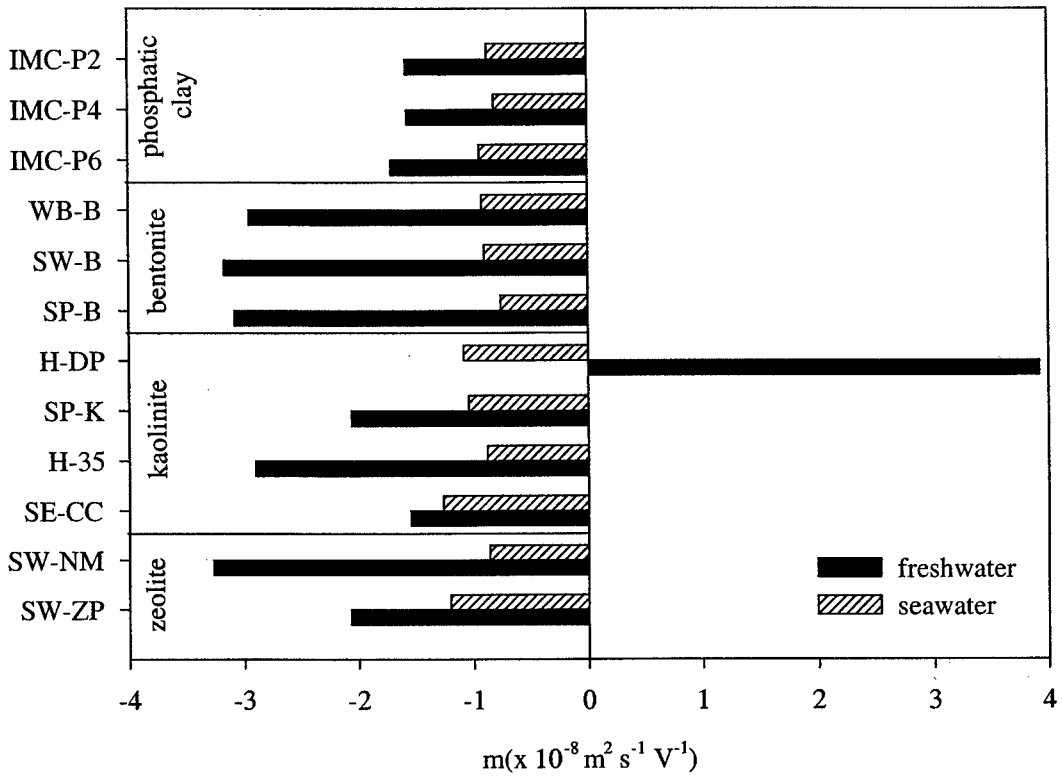
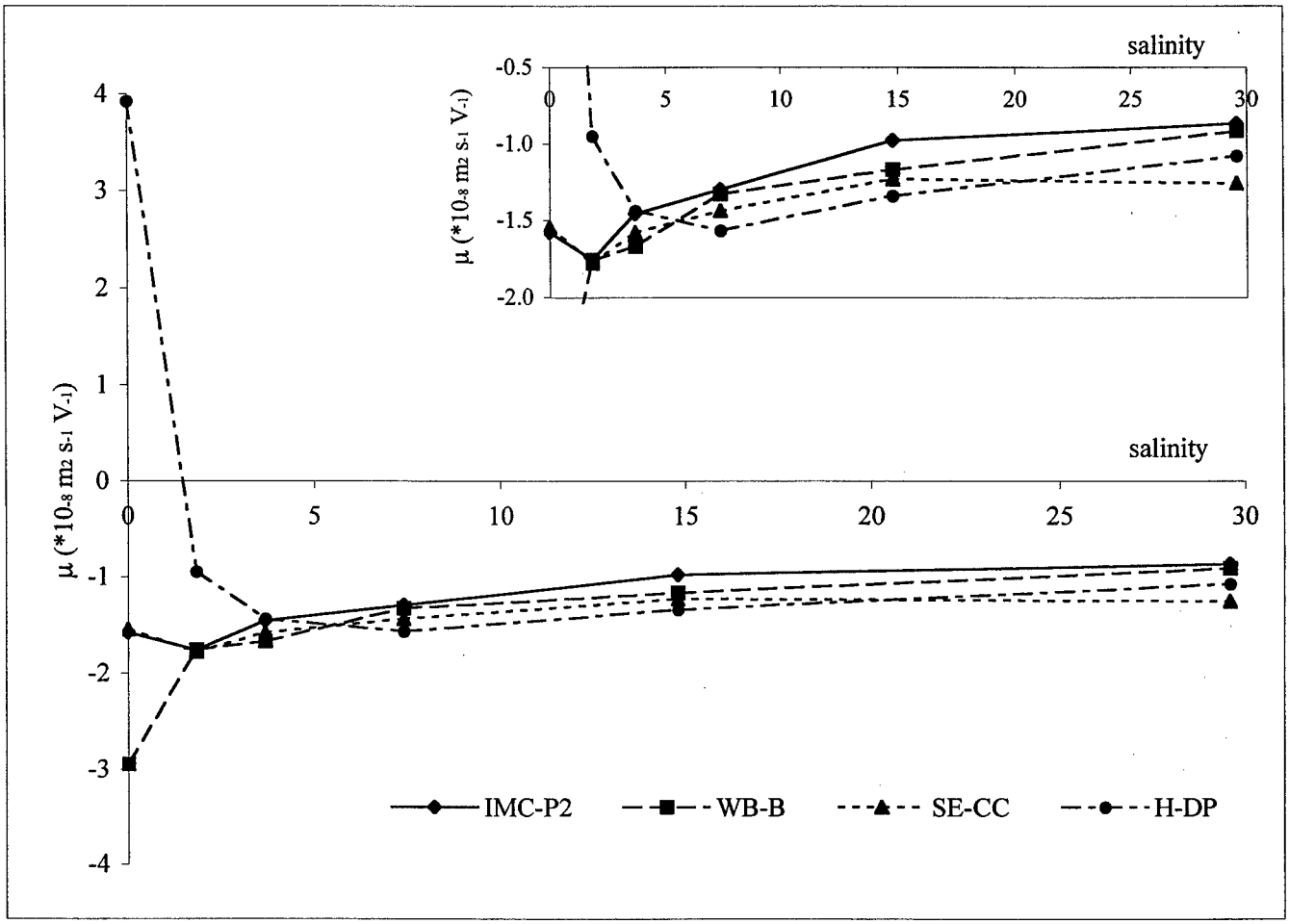

IMC-P2
IMC-P4
IMC-P6
WB-B
SW-B
SP-B
H-DP
SP-K
H-35
SE-CC
SW-NM
SW-ZP
phosphatic clay
bentonite
kaolinite
zeolite
freshwater
seawater
-4
-3
-2
-1
0
1
2
3
4
m(x 10^-8 m^2 s^-1 V^-1)

Table 4-5. Dilution study. Electrophoretic mobility (EPM) and zeta potential (ζ) of four clays minerals suspended in media with increasing salinity. The media were produced by diluting natural seawater (Vineyard Sound, MA) with distilled/ deionized water. The clay suspensions were allowed to equilibrate overnight in the medium with frequent agitation before the measurements were taken.

Salinity (ppt)	Viscosity (centipoise)	IMC-P2 phosphatic clay			WB-B bentonite			SE-CC kaolinite			H-DP treated kaolinite		
		EPM (10-8 m^2/V s)	*ZP* (mV)	pH	*EPM* (10-8 m^2/V s)	*ZP* (mV)	pH	*EPM* (10-8 m^2/V s)	*ZP* (mV)	pH	*EPM* (10-8 m^2/V s)	*ZP* (mV)	pH
0.000	0.8899	-1.6	-20.3	6.66	-3.0	-37.8	8.00	-1.6	-19.8	5.94	3.9	50.2	5.70
1.849	0.8943	-1.8	-22.7	7.11	-1.8	-22.6	7.03	-1.8	-22.9	7.16	-0.95	-12.3	7.04
3.698	0.8979	-1.5	-18.8	7.45	-1.7	-21.6	7.65	-1.6	-20.4	7.67	-1.4	-18.6	7.48
7.397	0.9049	-1.3	-17.0	8.22	-1.3	-17.4	8.37	-1.4	-18.8	8.38	-1.6	-20.4	8.30
14.794	0.9185	-0.98	-12.9	8.15	-1.2	-15.4	8.16	-1.2	-16.2	8.18	-1.3	-17.7	8.30
29.588	0.9458	-0.87	-11.9	8.21	-0.92	-12.5	8.25	-1.3	-17.1	8.26	-1.1	-14.7	8.27

Figure 4-3. Dilution study. Electrophoretic mobility (μ) of four clays minerals suspended in media with increasing salinity. The media were produced by diluting natural seawater (Vineyard Sound, MA) with distilled/deionized water. The clay suspensions were allowed to equilibrate overnight in the medium with frequent agitation before the measurements were taken. Inset: enlargement of the graph between -0.5 to -2.0 x 10^{-8} m^2 s^{-1} V^{-1}. IMC-P2 = phosphatic clay, WB-B Wyoming bentonite, SE-CC = Southeastern Crown Clay kaolinite, and H-DP Huber polymer-treated kaolinite (cationic).


salinity
μ (*10-8 m2 s-1 V-1)
-0.5
-1.0
-1.5
-2.0
0
5
10
15
20
25
30
salinity
μ (*10-8 m2 s-1 V-1)
4
3
2
1
0
-1
-2
-3
-4
IMC-P2
WB-B
SE-CC
H-DP

while the values reported here ranged from -2.5 to -7.7 mV at pH values from 8.19 to 8.95. The aggregation of algae was not observed despite their low EPM values. One explanation may be the steric stabilization of the particles by surface organic molecules (O'Melia and Tiller, 1993; Stumm, 1993). As for the motile species, the low aggregation rates may be explained by their ability to pull apart should attachment occur.

Since microalgae require light for growth, these organisms have developed a number of mechanisms to maintain their vertical position in the water column, especially near the surface (Walsby and Reynolds, 1980): cell motility, increase surface area by spines and protrusions, biosynthesis of more buoyant molecules (e.g. lipids), and the exchange of heavy ions for lighter ones. I propose that steric stabilization of marine algal cells may provide a mechanism to avoid aggregation and loss via settling. On the one hand, the various biochemical constituents of the cell membrane and the outer surfaces of the organism have important structural and regulatory functions. However, it is conceivable that these same molecules can also serve as a means of controlling the vertical position of the cell in the water column by minimizing the potential negative effect of aggregation.

Based on these results, the zeta potentials for the algae tested were small and very similar. It should be noted that in examining the removal efficiency of algae using clays, the removal rates were examined with respect to their zeta potential. However, these organisms are differentially removed by the clay. For example, *Gymnodinium breve* and *Heterocapsa triquetra* are more readily removed by the Florida phosphatic clay then the other species. Nevertheless, there is no difference in the zeta potential between these two and *Aureococcus anophagefferens*, for example, which is poorly removed by the clay. In another comparison, the two species with the highest ζ (i.e. *Skeletonema costatum* and *Prorocentrum micans*) were both poorly removed by the phosphatic clay. If the expectation were that the correlation between ζ and removal efficiency with phosphatic clay were inversely related, then one would predict that the species with the lowest ζ (i.e. *Chaetoceros simplex* or *Thalassiosira weisflogii*) would be removed most effectively. This was not the case (Chapter 3). Both of these species were poorly removed. Therefore, the measurement of algal zeta potential was not informative with

respect to why a given clay mineral would preferentially remove one species over another.

The measurement of zeta potential does not necessarily reflect of the amount and quality of organic matter on the surface, which may be the level at which the nature of the algal surface needs to be examined. For example, Cho et al. (1999) used various labelled lectins (i.e. chemicals specific to carbohydrates) to demonstrate that particles of given sample of yellow loess preferentially associated with certain types on carbohydrates on the cell surface of *Cochlodinium polykrikoides*. In addition, "living particles" such as cells are dynamic. Depending on many environmental factors, such as light or nutrient availability, marine algae may change their surface properties by changing not only the quantity of organic materials but also the quality (type of biochemical molecules) they discharge. Finally, the attachment of clays to the organism surface may be mediated by other organic particles that occur outside of the cell such as transparent exopolymers (TEP) (Jackson, 1995; Passow and Alldredge, 1995). These particles are formed in the surrounding medium from dissolved organic matter and attach to other particles which allow them to adhere. Since many removal studies were conducted in water used in culturing, organic matter for TEP production may be high, and this alternative model may occur.

Clay minerals and dilution experiment. The electrophoretic mobility values of the clay minerals from the present study were consistent with earlier observations in fresh-water systems (van Olphen, 1963; Bayne and Lawrence, 1972; Stumm and Morgan, 1996). Moreover, the higher zeta potential and stability of the bentonites also confirmed the observations of Whitehouse et al. (1960), Postma (1967), and Hahn and Stumm (1970) regarding the slower deposition of these clays relative to other clay minerals. Regarding the influence of clay surface properties on cell removal, Shirota (1989) suggested that the high removal ability of montmorillonites for algal cell relative to kaolinites is due to the high ion-exchange and adsorptive capacity of its three-layered structure over the two-layered structure of kaolinite.

In the present study, the only positive charge was found in the kaolinite H-DP, a clay that was pre-treated with cationic polymers. Therefore, this finding was expected. In a recent report (Sengco et al., 2001), this polymer-treated kaolinite, unlike the other

kaolinites tested, was one of the most effective at removing *Gymnodinium breve*. Its effectiveness was attributed to the presence of a positive charge which would make it more likely to adhere to the negative charges of the cell surface as verified here.

The Florida phosphatic clays displayed relatively low mobility and ζ. Despite the abundance of montmorillonite in these samples (Barwood, 1982), these values were much lower than those found for pure montmorillonite (bentonite) which showed the highest negative values. These results may be explained by the high Ca^{2+} ion content of the water used to produce the phosphatic clay slurry (Barwood, 1982; Bromwell, 1982). These bivalent ions are very effective at causing destabilization of clay suspensions (Edzwald et al., 1974), more so than monovalent ions like Na^{+}.

In seawater, however, the charge of every clay surveyed was negative and the electrophoretic mobilities and the ζ were uniform despite their wide range of values in freshwater and their mineralogy. Certainly, the reduction in EPM may be attributed to high adsorption of ions in the seawater environment, leading to the reduction in double layer thickness. In addtion, this phenomenon has been reported numerous times and has been explained as the adsorption of soluble, surface reactive, electronegative organic matter onto the clay surface (Neihof and Loeb, 1974; Hunter and Liss, 1979; Hunter, 1980; Hunter and Liss, 1982; Loder and Liss, 1985). These substances are often called humics. In several of these studies, various types of particles with known charges (positive, negative and neutral) were exposed to increasing amounts of estuarine or natural seawater. Even with very small quantities of seawater, these positive and strong negative charges are reduced to a tight range of electronegative values. Some reported values of electrophoretic mobility were -0.6 to -2.0 x 10^{-8} m^2 s^{-1} V^{-1} (Hunter and Liss, 1979), -0.65 x 10^{-8} m^2 s^{-1} V^{-1} (Hunter, 1980), -0.7 to -2.0 x 10^{-8} m^2 s^{-1} V^{-1} (Loder and Liss, 1985). The mobility measurements in the present study were well within this range: -0.76 to -1.3 x 10^{-8} m^2 s^{-1} V^{-1}. The consequence of this phenomenon may be seen in the reduction in the differential aggregation of clay minerals with increasing salinity as described by Whitehouse et al. (1960) and others. That is, the adsorption of these organic molecules may counteract inherent differences in charges (and stability) due to mineral structure and the effect of the medium (Neihof and Loeb, 1974; Hunter and Liss, 1979). Based on these results, the hypothesis that the variable effectiveness of different clay

minerals at removing a given algal species was associated with measurable differences in the surface charge and/or potential of the mineral in seawater may not be supported. If clays do display this pattern in seawater, and surface properties do become uniform despite mineralogy, then the important factor in the mutual aggregation with algal cells may be the clay's initial size and concentration. This will be tested in Chapter 5.

In Sengco et al. (2001), the clays were initially suspended in freshwater, then layered over the saline water column bearing the cells. Rapid aggregation was observed at the interface and large flocs entered the medium. In the dilution experiments reported here, the objective was to determine whether the "degree" of destabilization is different for the various clay minerals as the salinity gradually increased. This qualitative observation may provide additional information on the rate at which the particles enter the medium and their potential residence time in the water column.

In this study, the EPM and the ζ were quickly reduced as the salinity increased from 0 to just 1.8 ppt (Table 4-5, Figure 4-3). The largest shifts over this interval occurred in the bentonite WB-B and the cationic polymer-treated kaolinite H-DP. Both phosphatic clay IMC-P2 and the kaolinite SE-CC appeared relatively unaffected. At higher salinity values the electrophoretic mobilities and zeta potentials were restricted to an interval consistent with previous observations (e.g. Hunter and Liss, 1979). Drake (1976) predicted that the point of aggregation for minerals would occur at 2 salinity. Whitehouse et al. (1960) suggested a salinity range between 1 and 7 for destabilization. These results suggest that not only were the surface charges on clays rendered more uniform with increasing salinity, but that the process of altering the surface properties may be occurring very rapidly, with exposure to small amounts of natural seawater.

Based on this study, the determination of surface charge and zeta potentials as a simple diagnostic to predict the effectiveness of various clay minerals against a given species, or to predict which organisms would be removed more readily by a certain clay, was not feasible. Clays minerals are often flat, sheet-like structures and the charges on the minerals are not evenly distributed (Schwartz-Allen and Matijevic, 1974; Thomas et al., 1999). Depending on the properties of the medium (i.e. salt content, pH), the faces may be negatively charged but the edges may have slight positive charges due to broken bonds and lattice imperfections.In particle interactions, associations may occur which are

face to face, edge to edge or face to edge, leading to secondary, tertiary and higher order structures (van Olphen, 1963; Leussen, 1988). Furthermore, some properties of the clay suspension are affected by the type of associations present (e.g. viscosity) (van Olphen, 1963). More importantly, these associations can also affect the size and porosity of the aggregates being formed. Therefore, the differences in removal efficiency among clays may be influenced by how these clay-clay associations are formed as the salinity and pH increases. This information cannot be gleaned, from charge or electrokinetic data.

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CHAPTER 5

The Kinetics of Clay-Algae Aggregation and Settling: Laboratory Experiments with the Red-Tide Organism, *Karenia brevis*, and Three Clay Minerals

Abstract

The kinetics of aggregation and settling were studied in systems containing clay minerals alone (0.150 g L^{-1}), and in combination with the red tide organism, *Karenia brevis* (at 5000 cells ml^{-1}). Three clays with varying removal abilities against the organism were chosen: IMC-P2 phosphatic clay, WB-B bentonite and H-35 kaolinite. Data were collected by taking simultaneous measurement on a spectrophotometer (for clay mass) and a fluorometer (for cell mass). In seawater without *K. brevis*, kaolinite and phosphatic clay added to the surface aggregated rapidly and transitioned to settling within 20 min. Aggregates were transported from the upper layer between 40 to 60 min. By comparison, the aggregation rate of bentonite was very slow and produced only a few large flocs during the first 90 min of the experiment (total 150 min). Afterwards, the bentonite layer settled as one massive unit which swept through the water column. These kinetic patterns were also found when *K. brevis* was added. However, with cells present, the transition from aggregation to settling was delayed between 5 to 10 min for kaolinite and phosphatic clay. The initial bentonite dynamics were not affected by the presence of cells, but the deposition of material near the end of the time course occurred sooner. The kinetics of cell removal in the presence of the phosphatic clay closely followed the loss of clay mass. The final removal efficiency (RE) of *K. brevis* approached 100%. Initially, the kinetics of cell removal with kaolinite also followed the trajectory of clay loss, but 40 min after addition, cell removal slowed while clay loss continued. The RE with kaolinite was only 47%. In the case of bentonite, the kinetics of cell removal also paralleled clay flux, although the overall process was slow and limited by the aggregation rate of the clay. Nevertheless, the RE of *K. brevis* approached 97%. The kinetics of aggregation and settling for all clay-cell combinations were modelled as a first order equation process. These results are consistent with previous data regarding the removal ability of these three clay samples. However, the kinetics showed that the removal process is dynamic and distinctive for each mineral type. Moreover, depending on the mineral type, the removal of algae may be accomplished not only by mutual aggregation and settling, but by capture (or sweep-floc process) by larger clay aggregates falling through the medium.

Introduction

Recently, the use of clay minerals as a means of controlling harmful algal blooms (HABs) has garnered much attention and study (Shirota, 1989; Yu et al., 1994a, 1994b; Bae et al., 1998; Sengco et al., 2001). HABs are aquatic phenomena resulting from the proliferation of certain species of microalgae, many of which threaten public health, industry, other aquatic organisms, and the quality of freshwater and marine environments. The control strategy using clays is based on the mutual aggregation between the algal cells and the mineral particles, leading to the formation of large agglomerates that eventually settle to the ocean floor (Shirota, 1989; Ittekot, 1993). In the process, the algae are quickly and effectively removed from the water column, thus greatly minimizing their potential impacts. This method has proven effective in both laboratory trials (Maruyama et al., 1987; Yu et al., 1995a; Sengco et al., 2001), and in a number of field applications in Japan (Shirota, 1989) and South Korea (Na et al., 1996). From a practical standpoint, the use of clay minerals also offers several important advantages. They are abundant, natural substances that are already present in aquatic systems, and therefore, may cause minimal collateral and environmental damage (Portman, 1970; Howell and Shelton, 1970; Jack et al., 1993). Clay minerals are also relatively inexpensive, available in large quantities and easy to handle during application. Given these considerations and its successful implementation, clay dispersal has become one of the most promising strate-gies being investigated for directly managing HABs (Anderson, 1997).

As stated, the chief process involved in cell removal with clays is co-aggregation. According to physicochemical concepts, aggregation can be divided into two sequential steps, namely transport and attachment (O'Melia and Tiller, 1993). Transport is a physical process that brings about particle collisions and is controlled by the hydrodynamics of the system and external forces such as gravity. The three primary mechanisms are Brow-nian diffusion, fluid motion (either laminar or turbulent) and differential sedimentation. McCave (1984) determined that certain mechanisms become dominant during specific intervals of particle sizes. For example, diffusion is important when particles are < 1 μm, while fluid motion begins to dominate for larger particles, depending on the shear rate. For the largest particles, differential sedimentation is critical until they are finally lost to the system by settling. Similarly, Hunt (1980a) identified such regions in

the particle spectrum and developed a model using a power law function to calculate the influence of each mechanism through a size distribution. In a system with flagellated organisms, like many of those that produce HABs, particle collisions may also be generated by swimming ability (Jackson and Lochmann, 1993). In a recent study, the various theoretical collision frequency coefficients were calculated and compared in a system containing algal cells and clay particles (Chapter 3). The results showed that collisions produced by cell motility were potentially more important than diffusion and fluid motion, especially during the early stages of aggregation when the cells were interacting with clay particles < 50 μm. In the experimental system, such collisions may be occurring near the surface where the freshwater clay slurry (predominantly small particles) first comes in contact with the seawater containing algal cells. Therefore, the swimming speed of the organism, together with its size, can be a factor in determining its propensity for removal by a given cohesive mineral.

After transport, attachment can occur between the particles. This process is determined by the chemical properties on the particle surface and the chemistry of the surrounding medium (e.g. pH, ionic strength). For instance, clay minerals usually carry an electronegative charge which is balanced by an atmosphere of positively-charged ions (i.e. counterions) from the medium. This creates the so-called double layer around each particle. In media with low ionic strength, the double-layer is relatively thick and the overlapping interaction of two such layers from approaching particles yields an electrostatic repulsion that prevents attachment. However, as the double layer compresses with increasing ionic strength, repulsion decreases and aggregation begins. Algal cells also carry a surface charge which is generated by the ionization of functional groups on various types of organic matter on their surface. A recent investigation (Chapter 4) suggested that the stability of algal suspensions may be controlled not by compaction of the double layer, but by the amount and arrangement of long-chained polymers or electrolytes on the surface (i.e. steric stabilization) (O'Melia and Tiller, 1993). In this case, the portions of these bound molecules extend into the medium beyond the double layer where they can interact with empty sites on other particles (leading to aggregation), or with similar molecules extending from other particles (leading to repulsion).

The aggregation between algal cells and clay minerals is a complex phenomenon. It is an example of a heterodisperse suspension (i.e. consisting of particles with different sizes), where the range can span from submicron (clay minerals) to tens of microns for the largest algal species. Differences between the particles also extend to their density, chemical composition (i.e. organic vs. inorganic), surface chemistry, and their behavior (i.e. passive vs. actively motile). Nevertheless, the same physicochemical concepts have been applied to describe their mutual aggregation, mostly in a qualitative fashion (e.g. Avnimelech et al., 1982; Degens and Ittekot, 1984; Alldredge and Silver, 1988; Shirota, 1989; Yu et al., 1994a). In the most current model, interparticle collisions are brought about by the same suite of mechanisms (Alldredge and Silver, 1988; Yu et al., 1995b). However, collisions via Brownian diffusion have often been excluded since this mechanism is less effective for algal particles > 1 μm. Krank and Milligan (1980) focused on the effect of flow rate on the size and shape of natural agglomerations of algal cells and minerals (i.e. marine snow). Afterwards, Avnimelech et al.(1982) and Leslie et al. (1982) proposed that the attachment of clay particles on the cell surface was mediated by surface-active organic polymers produced by the organisms. The association was confirmed by electron and light microscopy. Yu et al. (1994a) determined that surface repulsive forces between particles explain the binding affinity of two different clays on the organism surface over a range of pH values. Finally, the differential effectiveness of various clay minerals for a given species has been explained by the adsorptive properties of clays (Shirota, 1989) and the biochemical composition of the cell surface (Cho et al., 1999).

Studies on the kinetics of clay-algae aggregation and settling have been rare. For heterodisperse systems, second order kinetics have been found for the aggre-gation and vertical flux in marine systems (Hunt, 1980; Hunt and Pandya, 1984). In one study, Yu et al. (1995b) used a spectrophotometer to follow the progress of aggregation and found a second order relationship between total particle loss and initial concentration. The rate constant for aggregation varied with time, which they proposed was due to two distinct phases: initial clay to clay aggregation followed by clay to cell aggregation. They also equated the rate constant to the collision frequency coefficients generated by particle collisions through molecular diffusion and differential sedimentation, although the

calculations for the theoretical coefficients were not performed. There were two additional weaknesses in the analysis that were not addressed by these authors: (1) the model did not account for particle loss from particle settling, and (2) the detection system was not able to discriminate between clays and cells, making it difficult to determine the loss of each and the final removal efficiency of the organism.

The objective of this paper was to quantify the kinetics of clay-algae aggregation and settling. This investigation deals with the dynamics of the system instead of the physicochemical properties of the individual particles which have been the focus of many previous studies. The system consists of the bloom-forming species, *Karenia brevis*, and three different clay minerals which have been shown to have different removal ability against this organism: phosphatic clay (high removal), bentonite (high removal) and kaolinite (poor removal). In addition to the physical characterization of the system, mathematical models describing the both clay and cell flux will be developed.

Materials and Methods

Algal culture. *Karenia brevis* (= *Gymnodinium breve*, CCMP718) was grown in batch cultures using modified f/2-Si medium under conditions described by Anderson et al. (1999). Growth was monitored using in vivo fluorescence (Model 10-AU Fluorometer, Turner Designs, Sunnyvale, California, USA) calibrated against direct microscope cell counts. Calibration and kinetic experiments were performed using cultures in early to mid-exponential growth.

Clay samples and preparation. The mineral samples and some of their properties are listed in Table 5-1. Sodium bentonite (WB-B) and kaolinite (H-35) were provided as dry fine powders. Phosphatic clay (IMC-P2) was obtained as a freshwater slurry with a 16% solid content (m/m) based on gravimetric analysis (Sengco et al., 2001). To fractionate the clays, 5.0 g of dry clay powder and an equivalent mass of wet phosphatic clay were suspended in 1 l of distilled/deionized water (DDI) with constant mixing. Hinder-ed settling was not observed. The slurry was then placed into a one-liter graduated cylinder where particles > 50 μm were allowed to settle for 4 min. Assuming a specific gravity of 2.00 for the clays and 20°C temperature, the supernatant was carefully decanted down to 32.8 cm from the surface, rediluted to 1 L with DDI and mixed

Table 5-1. Clay mineral samples for kinetic experiments. The reported specific gravity data were taken from the material safety data sheet for each clay. Electrophoretic mobility (EPM) was determined using a ZetaPALS system (Phase Analysis Light Scattering, Brookhaven Instruments Corporation, Holtsville, NY) (see Chapter 4). Freshwater (0 ppt) consisted of distilled/deionized water: density = 0.9971 g cm^{-3}, viscosity = 0.893 cp, pH = 7.41-7.83, temperature = 25C. Seawater (29.6 salinity) was taken from Vineyard Sound, MA: density = 1.0193 g cm^{-3}, viscosity = 0.946 cp, pH = 8.34-8.36, temperature = 25°C. *The minerals listed are the two most commonly found in phosphatic clay, making up to two-thirds of the total mass. The remaining part consists of several other mineral and non-mineral constituent (Barwood, 1984).

Clay	Trade name	Mineral composition	Clay Company	reported dry specific gravity	electrophoretic mobility (EPM) ($\times 10^{-8}$ m^2 s^{-1} V^{-1}) salinity = 0	salinity = 29.6
IMC-P2	phosphatic clay	primarily smectite and carbonate-fluorapatite*	IMC Phosphates, Inc.	2.9	-1.6	-0.87
WB-B	Wyoming bentonite	sodium bentonite	Wyo-Ben, Inc.	2.5	-3.0	-0.92
H-35	Huber kaolinite	kaolinite	J.M. Huber Corporation	2.6	-2.9	-0.88

thoroughly. The suspension was again placed into a graduated cylinder where particles > 10 μm were allowed to settle for 95 min. The supernatant was collected down to a depth of 31 cm. To determine the solid content of all three fractions (i.e. > 50 μm, < 50 μm to > 10 μm, and < 10 μm), 5-ml aliquots were placed in triplicate beakers, dried overnight at 105°C, and analyzed gravimetrically. Only the fraction containing particles < 10 μm were used in all experiments.

Calibration experiments. To determine the mass of clay minerals and algal cells simultaneously during the kinetic experiments, a spectrophotometer (Shimadzu UV-VIS) and fluorometer (Turner Designs 10-AU) were calibrated first using known amounts of either type of particle, and for mixtures containing various proportions of both. The spectrophotometer was used primarily to monitor the mass of clay minerals (i.e. absorbance at 500 nm) (Hunt and Pandya, 1984). The fluorometer was used to track the mass of algal cells (Avnimelech et al., 1982).

First, concurrent measurements using both instruments were made for clay alone at final concentrations of 0, 0.015, 0.045, 0.075, 0.113 and 0.150 g l^{-1} (11 ml final volume). The suspensions were placed in borosilicate test tubes (1.4 cm inner diameter, 15 cm tall). Each sample was vortexed strongly for 2 to 3 sec before taking measurements. For the spectrophotometer, 2-ml aliquots were placed in plastic cuvettes. For the fluorometer, the entire tube was placed into detector. Next, concurrent measurements were taken for each of the previous clay loadings which were also combined with the following cell concentrations (in cells ml^{-1}, the corresponding dry mass of algae in parenthesis): 0, 500 (2.2 mg l^{-1}), 1250 (5.5 mg l^{-1}), 2500 (11 mg l^{-1}), 3740 (16.5 mg l^{-1}), and 5000 (22 mg l^{-1}). Calibration curves for each instrument were generated using least-squares fit on the data. Each curve was used for the appropriate combination of cells and mineral type.

Kinetic experiments: In the first set, the kinetics of clay aggregation and settlement were studied in the absence of algal cells. 10 ml of autoclaved, 0.20-μm cartridge-filtered seawater (salinity = 29.6, pH = 8.32, 25°C, Vineyard Sound, MA) was placed in 18 pairs of borosilicate test tubes. 1 ml of fractionated clay (< 10 μm) was carefully layered over the water column using an air-displacement pipet (0.150 g l^{-1} final concentration in 11 ml) (Figure 5-1). Aggregation and settling proceeded in a quiescent system (Sengco et al., 2001). At each time point, a pair of tubes was sacrificed beginning

at t = 0 min (initial), then at 5-min intervals up to 60 min, followed by 10-min intervals up to 90 min, and finally 30-min intervals until the experiment terminated at 150 min (final). The samples were taken by withdrawing the upper 10 ml (6.3 cm) from each tube and placing them into clean tubes. Care was taken to avoid disturbing the water and pellet in the remaining 1 ml. The supernatants were then vortexed strongly for 2 to 3 sec, and the measurements for the spectrophotometer and fluorometer performed as previously described. Photographs of the experimental system were taken at each time point to document progress. This procedure was repeated for all three clay minerals. In the second set of experiments, 10 ml of *Karenia brevis* cultures were used in place of the filtered seawater. The initial cell concentration was 5000 cells ml^{-1} (22 mg l^{-1}). All experiments were conducted at room temperatures (20-25°C)

Modelling aggregation/settling and cell removal. After describing and characterizing each of the clay-algae systems in a conceptual model, the choice of suitable mathematical expressions was made given the observations. The primary focus of this effort was the cell removal from the water column. The models and the parameters will be presented in the following section.

Results

Calibration. For this study, a method had to be developed to track the change in mass concentration for two different types of particles in the aggregating system. In previous reports, the spectrophotometer and fluorometer have been used individually for clay suspensions and cell cultures, respectively. However, it was necessary to evaluate how the measurements given by each instrument would be affected when the samples contained particles different from those it was used to detect.

The calibration curves for the spectrophotometer are presented in Figure 5-2. The slope of each curve for the three clays minerals varied with the kaolinite showing the highest value (steepest), followed by the phosphatic clay and finally the bentonite. The r-squared values were high. In cases where each clay loading was mixed with increasing amounts of *Karenia brevis*, the average value and the standard deviation were calculated (Fig. 5-2B, D, F). In comparing systems without and with *K. brevis*, the slope for IMC-P2 phosphatic clay changed by 4%, for WB-B bentonite by 6% and for H-35 kaolinite by

Figure 5-1. Test-tube reactor for studying the kinetics of clay-algae aggregation and settling. Height = 15 cm, inner diameter = 1.4 cm. (A) Initial conditions showing the buoyant freshwater clay slurry at the surface. Clay destabilization in seawater and rapid aggregation occurred at the freshwater-seawater interface. (B) Final condition after 2.5 hours. Clay-cell pellet on the bottom, below the sampling depth.

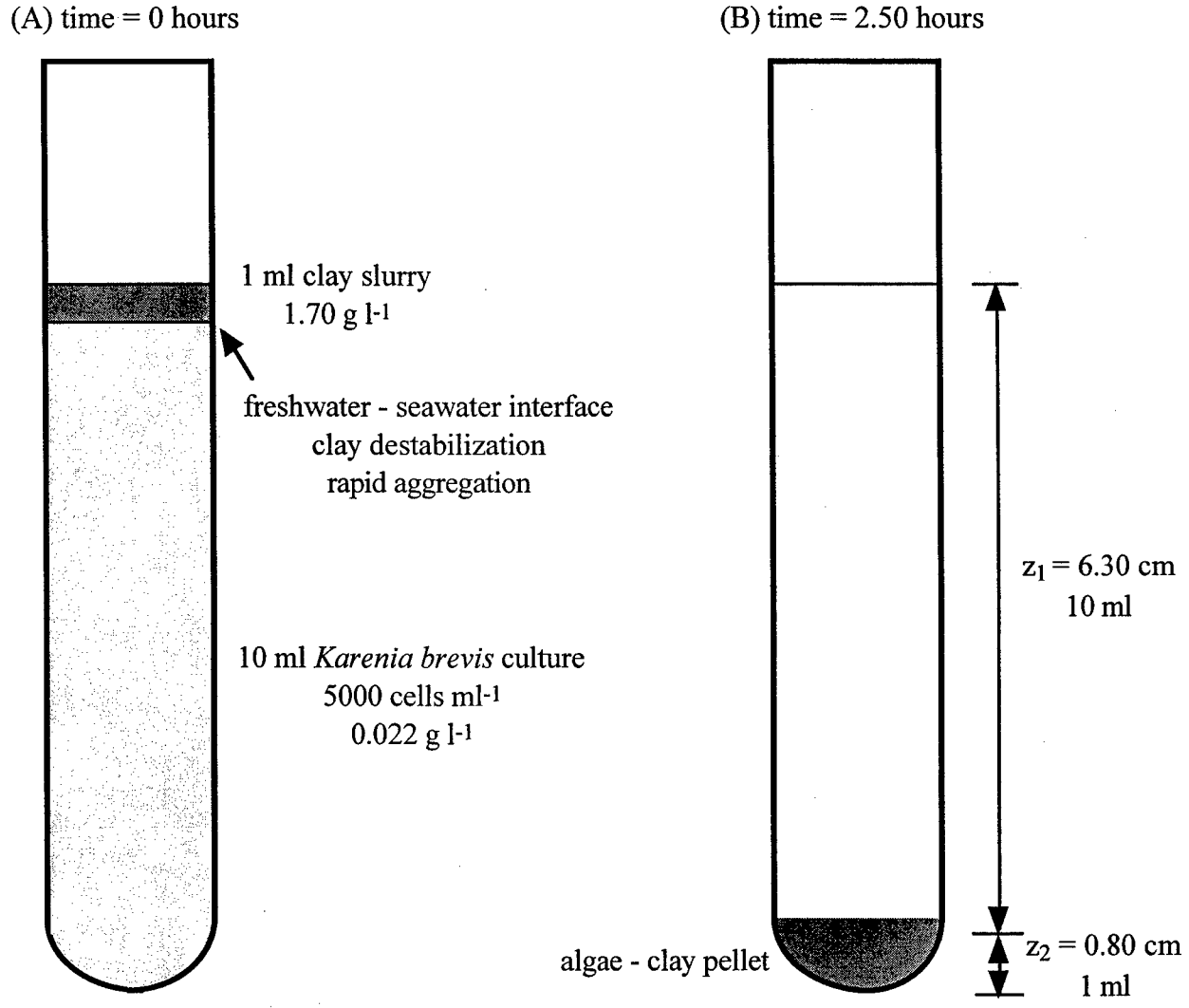

(A) time = 0 hours
(B) time = 2.50 hours
1 ml clay slurry
1.70 g l-1
freshwater - seawater interface
clay destabilization
rapid aggregation
10 ml Karenia brevis culture
5000 cells ml-1
0.022 g l-1
algae - clay pellet
z1 = 6.30 cm
10 ml
z2 = 0.80 cm
1 ml

Figure 5-2. Sprectrophotometer calibration curves for three clay minerals, with and without *Karenia brevis*. (A)-(B) IMC-P2 phosphatic clay, (C)-(D) WB-B sodium bentonite, (E)-(F) H-35 kaolinite. The three panels to the left represent clays alone and the three panels to the right represent a well-mixed suspension of clays and cells. Error bars represent the standard deviation about the mean when all the measurements at a given clay loading, plus a range of cell concentrations, were combined. The best fit line was determined from least squares.

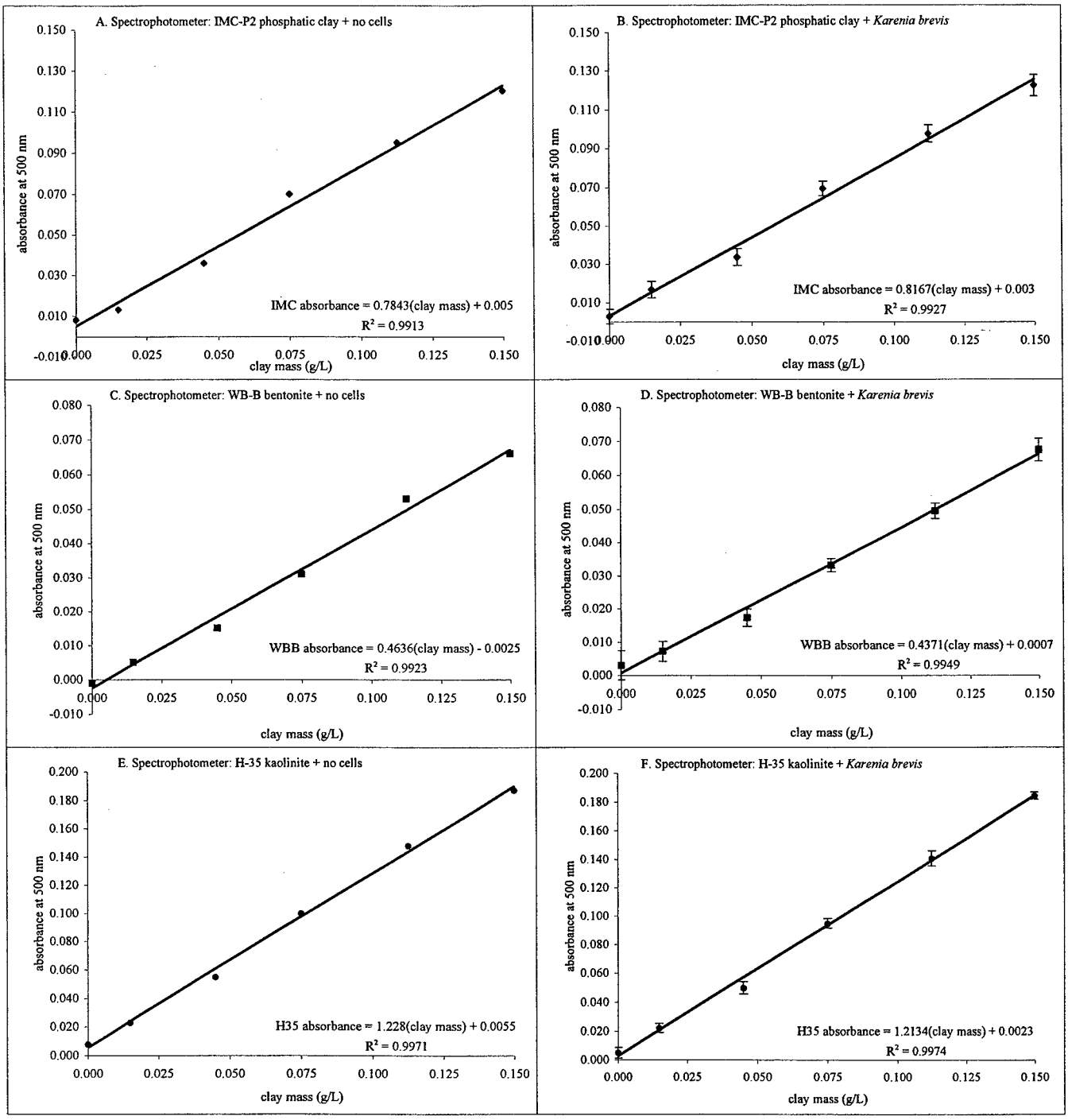
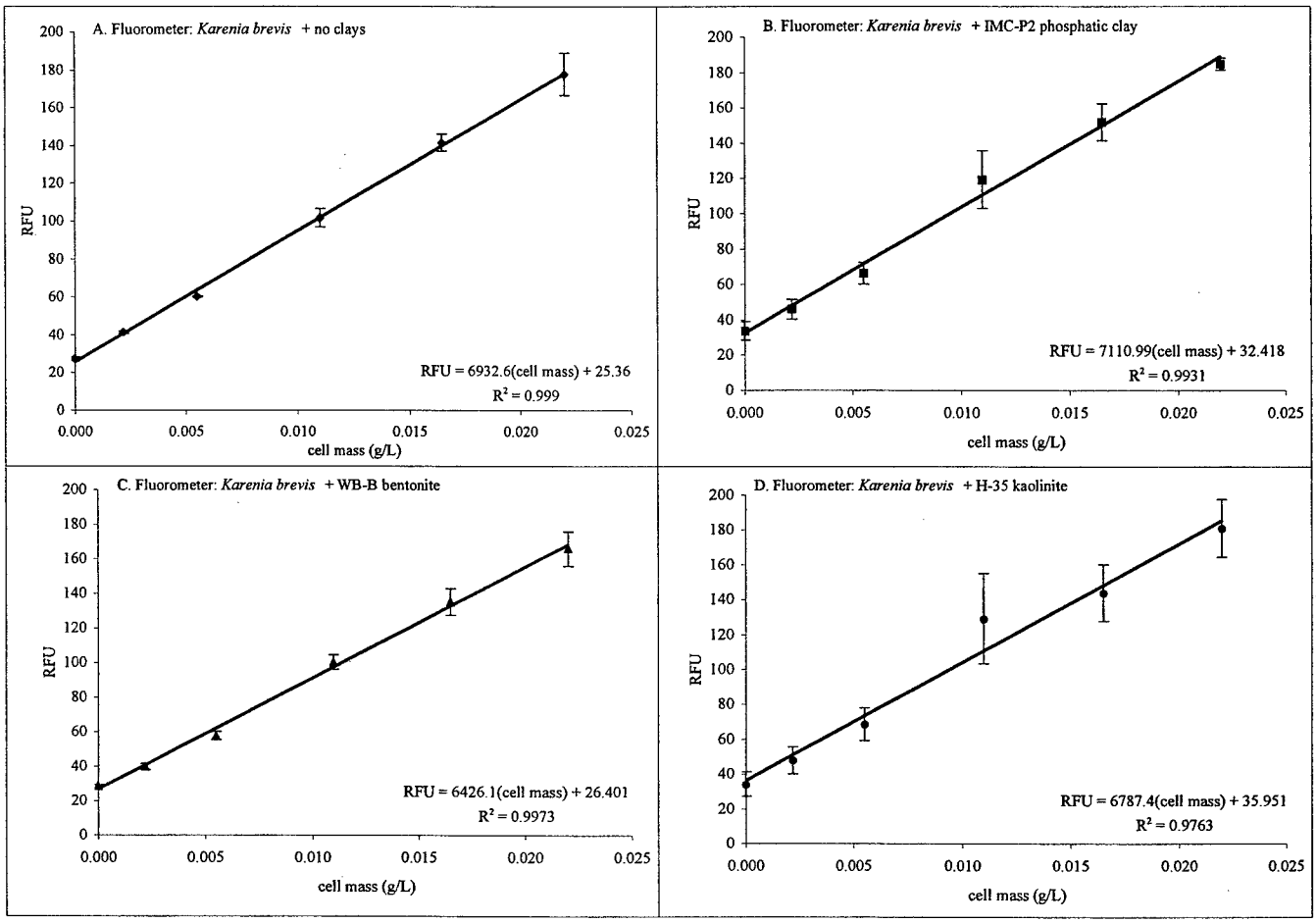

A. Spectrophotometer: IMC-P2 phosphatic clay + no cells
absorbance at 500 nm
clay mass (g/L)
IMC absorbance = 0.7843(clay mass) + 0.005
R² = 0.9913
B. Spectrophotometer: IMC-P2 phosphatic clay + Karenia brevis
absorbance at 500 nm
clay mass (g/L)
IMC absorbance = 0.8167(clay mass) + 0.003
R² = 0.9927
C. Spectrophotometer: WB-B bentonite + no cells
absorbance at 500 nm
clay mass (g/L)
WBB absorbance = 0.4636(clay mass) - 0.0025
R² = 0.9923
D. Spectrophotometer: WB-B bentonite + Karenia brevis
absorbance at 500 nm
clay mass (g/L)
WBB absorbance = 0.4371(clay mass) + 0.0007
R² = 0.9949
E. Spectrophotometer: H-35 kaolinite + no cells
absorbance at 500 nm
clay mass (g/L)
H35 absorbance = 1.228(clay mass) + 0.0055
R² = 0.9971
F. Spectrophotometer: H-35 kaolinite + Karenia brevis
absorbance at 500 nm
clay mass (g/L)
H35 absorbance = 1.2134(clay mass) + 0.0023
R² = 0.9974

Figure 5-3. Fluorometer calibration curves for *Karenia brevis*, with and without the three clay minerals (at 0.15 g l^{-1}). (A) *K. brevis* alone, (B) *K. brevis* with IMC-P2 phosphatic clay, (C) *K. brevis* with WB-B bentonite, and (D) *K. brevis* with H-35 kaolinite. The error bars represent the standard deviation about the mean when all the measurments for a given cell concentration, plus a range of clay concentrations, were combined. The best fit line was determined from least squares.


A. Fluorometer: *Karenia brevis* + no clays
RFU
cell mass (g/L)
RFU = 6932.6(cell mass) + 25.36
R^2 = 0.999
B. Fluorometer: *Karenia brevis* + IMC-P2 phosphatic clay
RFU
cell mass (g/L)
RFU = 7110.99(cell mass) + 32.418
R^2 = 0.9931
C. Fluorometer: *Karenia brevis* + WB-B bentonite
RFU
cell mass (g/L)
RFU = 6426.1(cell mass) + 26.401
R^2 = 0.9973
D. Fluorometer: *Karenia brevis* + H-35 kaolinite
RFU
cell mass (g/L)
RFU = 6787.4(cell mass) + 35.951
R^2 = 0.9763

1.2%. On careful inspection of the data, the highest possible error was found when clay loading was very low (e.g. 0.015 g l^{-1}) in the presence of algae (data not shown).

The calibration curves for the fluorometer are presented in Figure 5-3, with cells alone (Fig. 5-3A) and in combination with various amounts of clay at each cell concentration (Fig.5-3B, C, D). The slopes for each curve were very similar despite differences in the clays added. As before, the r-squared values were high. The greatest possible interference of clay particles was observed when cell concentrations were low to moderate (e.g. up to 20% in the presence of kaolinite, Fig. 5-3D). However, variability in the remaining measurements for the other clay-cell combinations was between 2 and 12%.

Kinetic experiments - Clays in seawater without *K. brevis*. The sequences of aggregation and sedimentation for the three clays in seawater in the absence of algae are shown in Figure 5-4. In all cases, the freshwater clay slurry was located at the surface at 0 min (initial). At 10 min, larger particles were seen at the bottom of the turbid surface layer, near the interface between freshwater and seawater. By 15 min, the kaolinite H-35 was the first to show settling, followed by the phosphatic clay IMC-P2 at 20 to 25 min. This pattern was clearly seen in the spectrophotometer data as well (Figure 5-5). Although it is difficult to ascertain from the photographs, the settling particles of kaolinite appeared smaller and rougher in apparent texture, than those of phosphatic clay which appeared somewhat larger and fluffier like marine snow (Alldredge and Silver, 1988). By 30 min, both kaolinite and phosphatic clay were generating particles from the turbid layer at a constant pace. Between 60 and 70 min, the production of large particles mark-edly decreased and only fine particles remained in the turbid layer. At 70 min, 87% and 72% of the initial mass of phosphatic clay and kaolinite, respectively, were already lost (Figure 5-5). Eventually, the small particles settled out by 150 min. Some kaolinite par-ticles and aggregates seemed to adhere to the walls of the test tube (Figure 5-4, 150 min).

For the bentonite WB-B, the process was very different. At 20 min, the formation of large flocs was observed near the freshwater-seawater interface as with the other two clays. However, rapid settling of these aggregates from the turbid layer was not observed. Instead, they continued to increase in size. By 25 min, only a few large individuals were falling out. These were difficult to see since they were translucent,

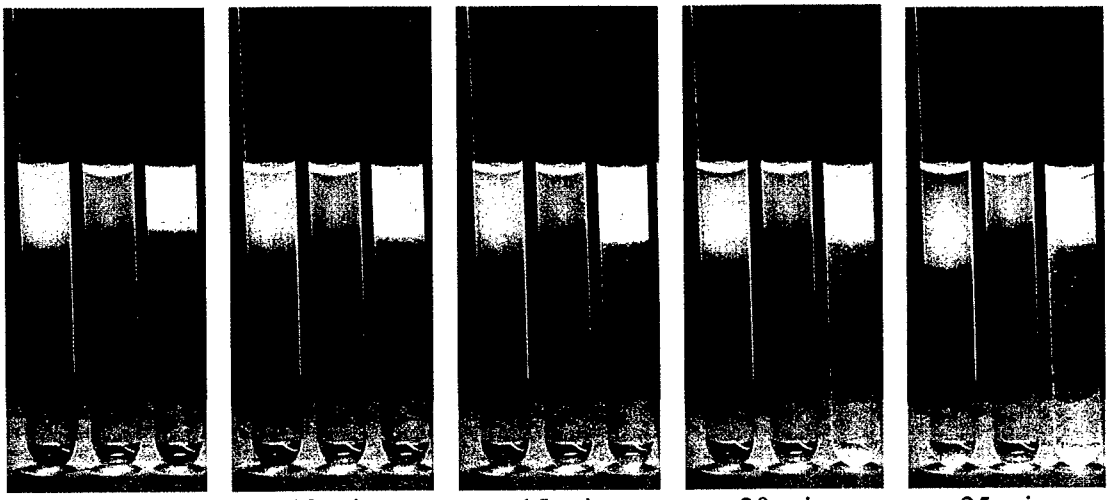
feathery and quite voluminous. At 70 min, only 27% of the clay mass was lost. Finally, the entire layer appeared to settle as one huge agglomerate by 90 min. The layer swept through the medium mostly intact and rested on the bottom by 150 min (Figure 5-4). This stage represented a 95% loss of initial mass.

Kinetic experiments - Clays with *Karenia brevis*. The kinetic data for the clays mixed with *K. brevis* followed the same general patterns as for clays alone. However, the presence of the algae appeared to alter the timing of the process for the phosphatic clay and kaolinite (Figure 5-6). The change was associated with the transition from clay aggregation to the onset of rapid settling. The delay was in the range of 5 to 10 min. Nevertheless, the kinetics appeared to be generally similar to the cell-free system. For the bentonite, the presence of *K. brevis* did not appear to have any effect during the initial stages. At 90 min, however, the massive deposition of the turbid layer seemed to occur faster with the addition of cells, so that by 120 minutes, the layer was already lost from the system (Figure 5-6B).

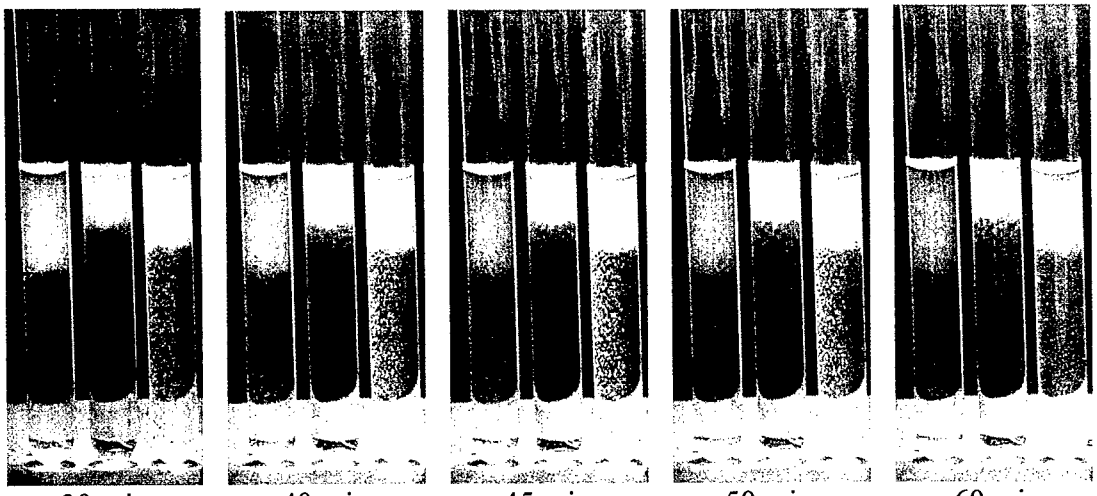
Focusing on the kinetic data for *Karenia brevis*, the patterns were quite different with each clay combination (Figure 5-7). For the phosphatic clay, the disappearance of *K. brevis* followed the loss of clay mass (Figure 5-7A). The fastest removal took place between 20 and 60 min, which marked the transition from aggregation to settling by the clay. The final removal efficiency (RE) was exceptionally high (100%). Similarly, the kinetics of cell removal appeared to coincide with the kinetics of cell loss in the kaolinite (Figure 5-7C). However, the two curves diverged at 40 min. Clay loss continued in the same manner as in the cell-free system, but cell flux slowed down markedly. The final RE of *K. brevis* was only 47% despite the reduction in clay mass by 77% during the course of the treatment.

In the case of bentonite, cell removal was steadily taking place even though the aggregation-settling rate of the clay was much slower (Figure 5-7B). From 5 to 90 min, the RE of *K. brevis* was at 71% while the clay loss was only 46%. After the massive deposition of the bentonite layer occurred, the cell RE was 97%, matching the value for phosphatic clay, and 97% of initial clay mass was lost. The loss due to sinking of *K. brevis* (control without clay added) ranged between 9% and 12% for all experiments.

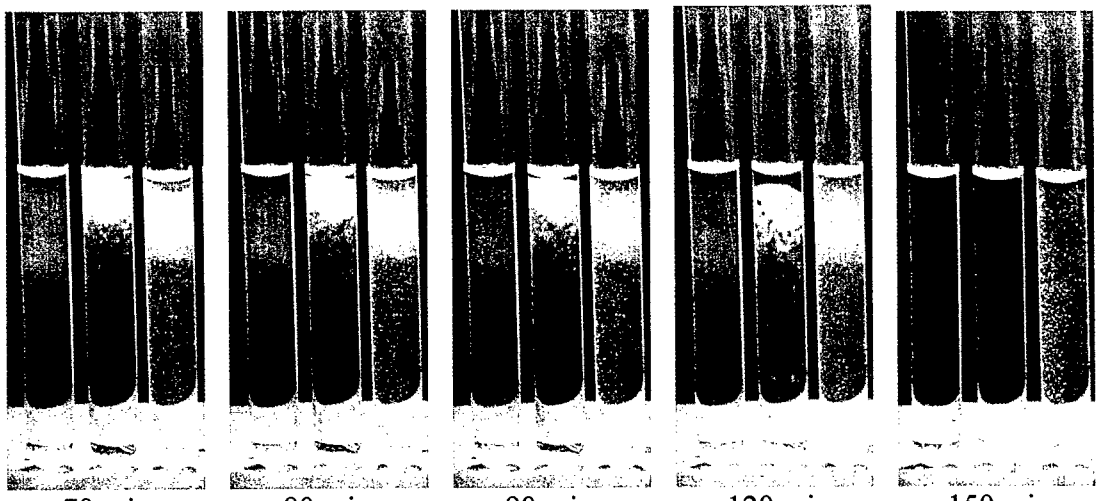
Figure 5-4. Photographs from the kinetic experiments of clay aggregation and settling. The panels show the progress of the aggregation and settling of the three clays through time over seawater (no cells). The first tube to the left contains IMC-P2 phosphatic clay, the middle tube WB-B bentonite, and the third tube H-35 kaolinite. The same appearance and sequence of events were documented in the case when *K. brevis* were present in the water column, although the exact onset of aggregation and sedimentation were slightly delayed (see Results).



0 min 10 min 15 min 20 min 25 min



30 min 40 min 45 min 50 min 60 min



70 min 80 min 90 min 120 min 150 min

Figure 5-5. Kinetics of aggregation and settling of clay minerals without *Karenia brevis*. The data were taken from spectrophotometer measurements (i.e. absorbance at 500 nm) which were then converted to mass concentration per volume by the appropriate calibration curves. The error bars represent standard deviation for duplicate samples at each time interval.

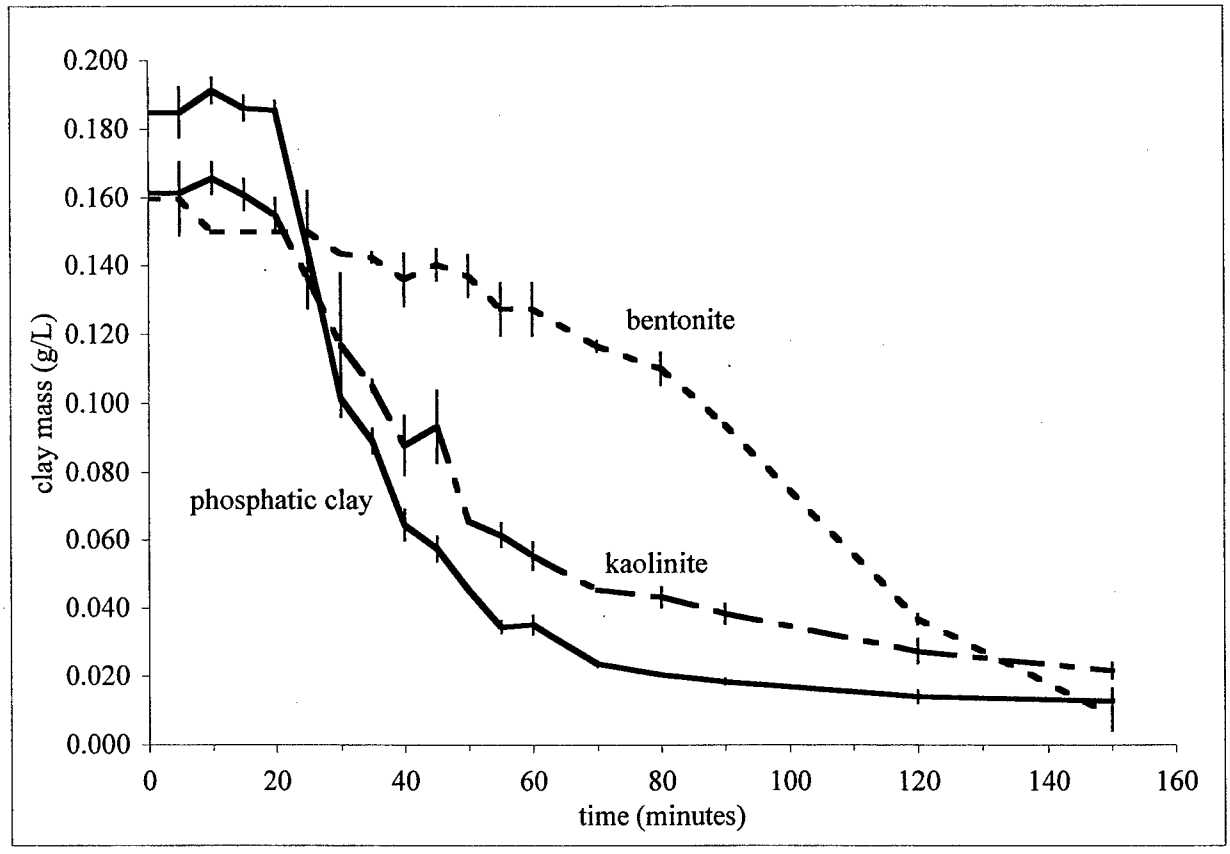

0.200
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
clay mass (g/L)
bentonite
phosphatic clay
kaolinite
0
20
40
60
80
100
120
140
160
time (minutes)

Figure 5-6. Comparison of the kinetics of aggregation and sedimentation of clays with and without *Karenia brevis*. Data were taken from spectrophotometeric measurements. Error bars represent the standard deviation for duplicate samples. (A) IMC-P2 phosphatic clay, (B) WB-B bentonite, (C) H-35 kaolinite.

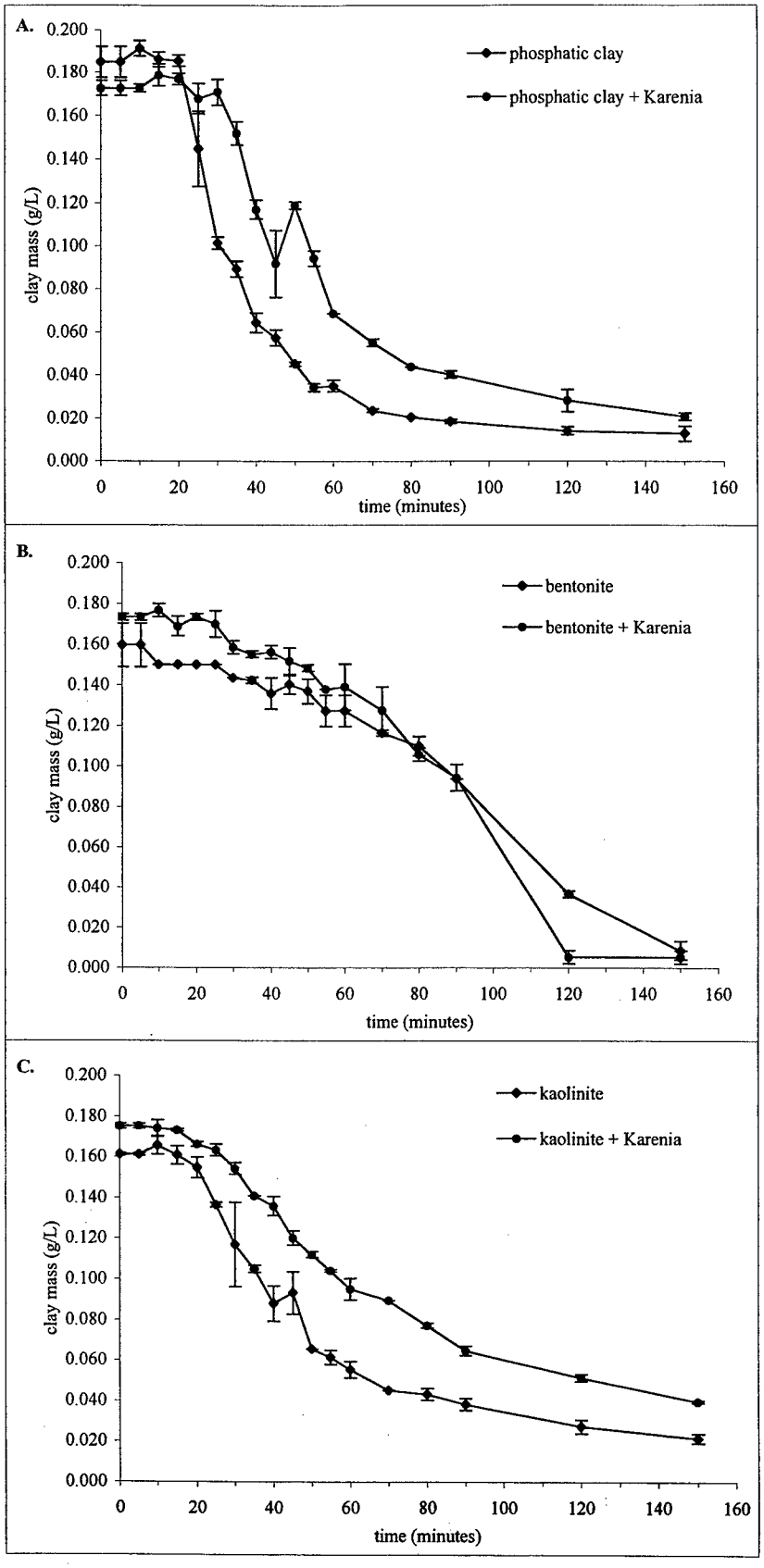

A.
phosphatic clay
phosphatic clay + Karenia
clay mass (g/L)
time (minutes)
B.
bentonite
bentonite + Karenia
clay mass (g/L)
time (minutes)
C.
kaolinite
kaolinite + Karenia
clay mass (g/L)
time (minutes)

Figure 5-7. Kinetics of aggregation and settling of *Karenia brevis* with clay minerals. (A) IMC-P2 phospahtic clay, (B) WB-B bentonite, and (C) H-35 kaolinite. The graphs simultaneously display the trends for clay mass from the spectrophotometer (absorbance at 500 nm), and cell mass from the fluorometer over time. The cell removal efficiency (RE) was calculated using the following equation: RE = (initial mass - final mass)/initial mass *100.

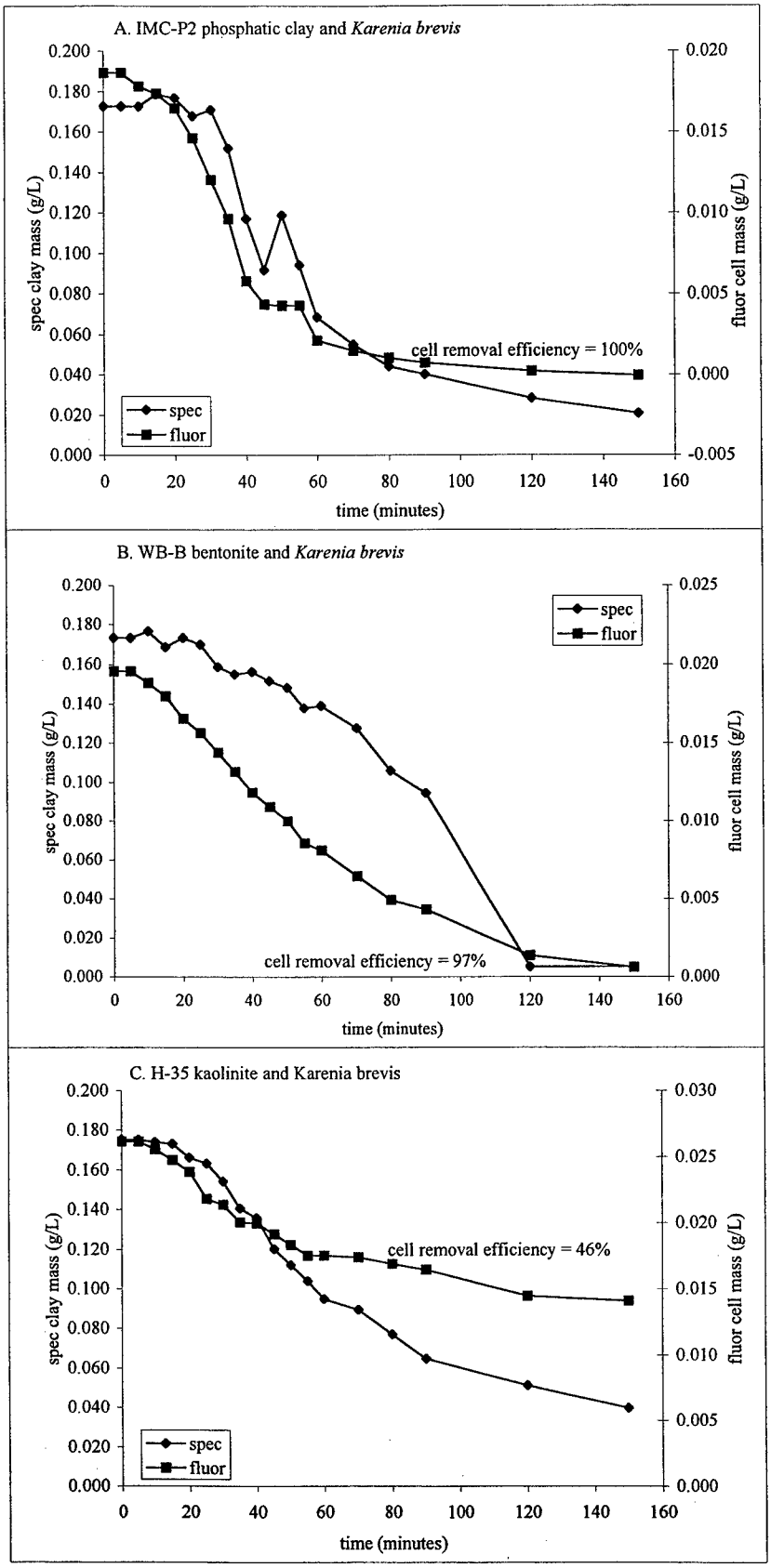

A. IMC-P2 phosphatic clay and *Karenia brevis*
spec clay mass (g/L)
fluor cell mass (g/L)
time (minutes)
cell removal efficiency = 100%
spec
fluor
B. WB-B bentonite and *Karenia brevis*
spec clay mass (g/L)
fluor cell mass (g/L)
time (minutes)
cell removal efficiency = 97%
spec
fluor
C. H-35 kaolinite and Karenia brevis
spec clay mass (g/L)
fluor cell mass (g/L)
time (minutes)
cell removal efficiency = 46%
spec
fluor

Figure 5-8. Total mass flux of *Karenia brevis* with clay minerals. The data represent the sum of the clay mass as determined by the spectrophotometer (absorbance at 500 nm) and the cell mass from the fluorometer. Error bars represent the standard deviation for duplicate samples.

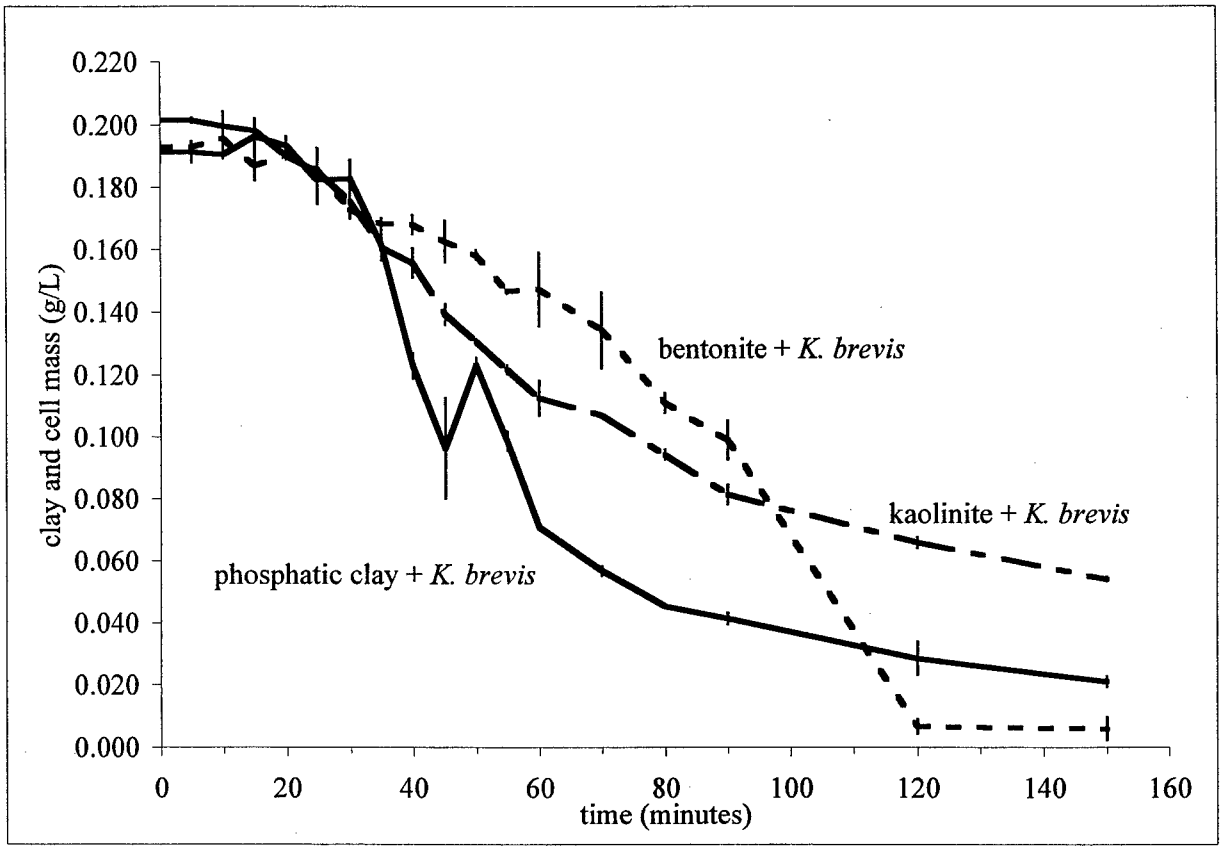

0.220
0.200
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
clay and cell mass (g/L)
0
20
40
60
80
100
120
140
160
time (minutes)
bentonite + K. brevis
kaolinite + K. brevis
phosphatic clay + K. brevis

Figure 5-9. Conceptual model of clay-cell aggregation and settling based on empirical observations. Zone 1: Clay slurry in freshwater at 0.15 g l^{-1} final clay loading (initial concentration 1.65 g l^{-1}). Zone 2: Freshwater-seawater interface. Region of clay destabilization, clay-clay aggregation, and initial contact with algal cells occur, leading to the formation of rapidly-sinking aggregates. Region where the rate limiting step in the process is taking place. Zone 3: Clay-cell aggregation and settling. Aggregates interact with algal cells and remove them by sweep-floc process. Zone 4: Deposition. The final outcome of the settling where the particles are lost to the system.

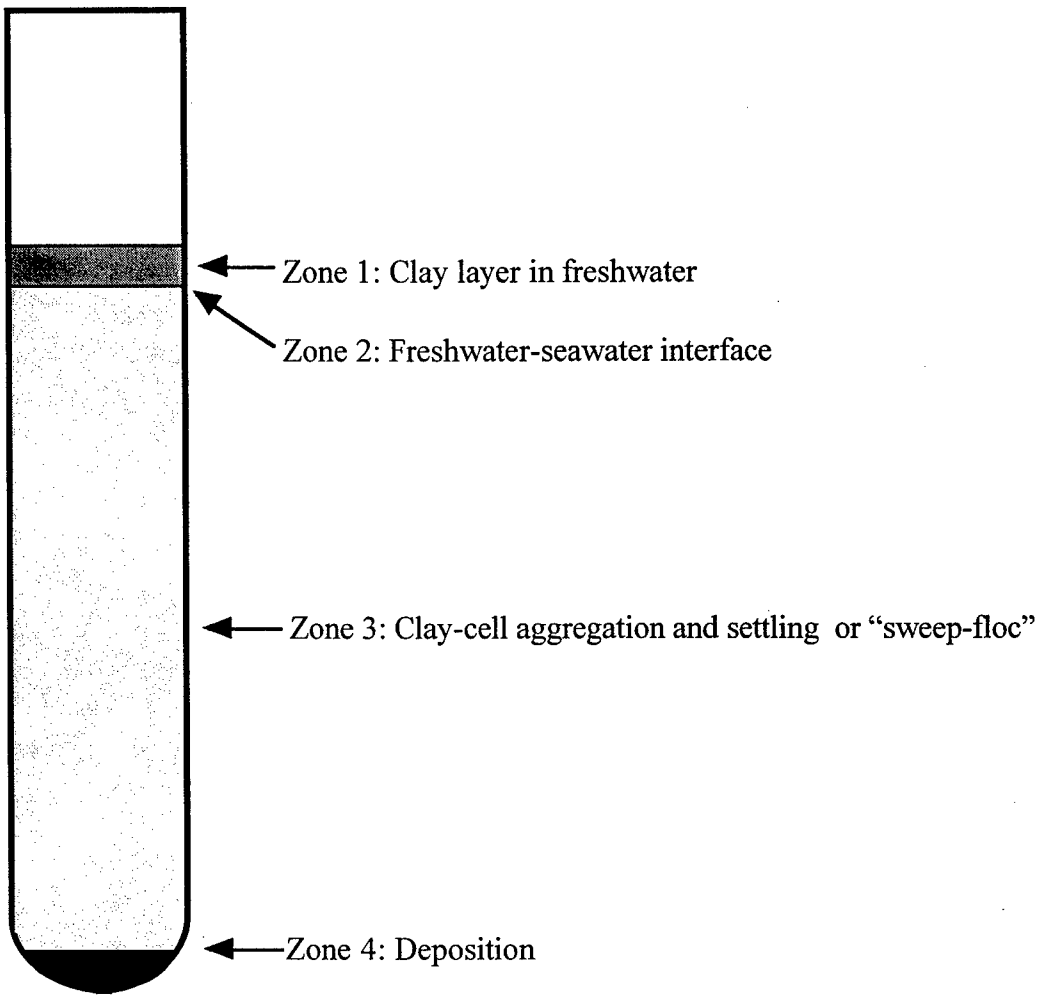

Zone 1: Clay layer in freshwater
Zone 2: Freshwater-seawater interface
Zone 3: Clay-cell aggregation and settling or "sweep-floc"
Zone 4: Deposition

Table 5-3. Model results from kinetic experiments using clay minerals and *Karenia brevis*. In the clay-algae system, clay mass was monitored using a spectrophotometer and cell mass was determined using a fluorometer. The total mass flux is the sum of clay and cell mass. The reaction order was chosen to reflect the experimental design and the physical description of the aggregation and settling phenomenon. The time intervals were selected over the period when the majority of *K. brevis* removal was taking place. The value for k represented the slope for integrated form of each function in Table 5-2. It was divided by C_o to give units of min^{-1}. Settling velocity was determined by multiplying k by the height of the water column (6.3 cm). The final removal efficiency (RE) was calculated for the 150-min duration of the experiment.

Clay and Algae System	time interval (min)	k (1/min)	curve fit r^2	settling velocity (cm/min)	% RE over time interval	final % RE
IMC-P2 phosphatic clay						
clay without cells	20 - 60	0.047	0.99	0.29	81.1	93.1
clay with cells	20 - 60	0.023	0.86	0.14	61.3	87.9
total mass flux	20 - 60	0.024	0.88	0.15	63.4	89.1
K. brevis with IMCP2	20 - 60	0.049	0.95	0.31	87.0	100.0
WB-B bentonite						
clay without cells	5 - 90	0.005	0.87	0.03	41.2	94.6
clay with cells	5 - 90	0.007	0.85	0.04	45.5	97.0
total mass flux	5 - 90	0.008	0.87	0.05	48.8	97.0
K. brevis with WB-B	5 - 90	0.019	0.99	0.12	71.9	97.0
H-35 kaolinite						
clay without cells	15-70	0.025	0.98	0.15	71.9	86.6
clay with cells	15-70	0.013	0.98	0.08	48.3	76.3
total mass flux	15-70	0.012	0.98	0.08	48.0	73.3
K. brevis with H-35	10-55	0.008	0.98	0.05	31.4	46.2

The total mass transport of *K. brevis* and clay minerals are combined and summarized in Figure 5-8. In general, the mass flux followed the trend for clay minerals (i.e. the spectrophotometer readings) since the amount of clays was about 10 times more than the mass of cells in this experiment.

Conceptual model: In this study, the design and performance of the experiment considered the way in which the clay would eventually be dispersed in a field treatment of a HAB (Figure 5-1). For instance, there would be an initial depth separation between the applied clay layer and the bulk of the cell suspension. Presumably, clay to clay aggregation would begin at the surface with a minor amount of clay-cell aggregation until flocs form that are dense or large enough to sink. This stage is the first potential bottleneck or rate-limiting step in cell removal. Afterwards, the cells in the bulk of the fluid would come in contact with these downward-moving clay aggregates. The cell removal process would then be governed by several factors including the size of the clay flocs, their velocity, the size of the organisms and the "stickiness" of the clays and the cells.

Based on the kinetic information from the clay-cell system, a conceptual model can be constructed (Figure 5-9). In Zone 1, the freshwater clay slurry in located at the surface. The lower relative density of the freshwater medium prevents mixing and allows the layer to remain intact. Moreover, the focusing of this layer at the surface allows the clay concentration to remain high, which forces aggregation forward. The degree of aggregation is this layer will also depend on the relative stability of the suspension. For example, based on electrophoretic mobility measurements, bentonite is more stable than kaolinite, while the phosphatic clays are the least stable.

In Zone 2, the interface between seawater and freshwater is located. Destabilization of the clay is occurring in this region leading to clay-clay aggregation and increasing settling rates. This is also the zone where the initial stages of clay-cell aggregation is taking place, especially between algal cells and intermediate-sized clay particles resulting from aggregation. Based on the kinetic data, the rate-limiting step in the entire process appears to be located in this zone and is controlled by the aggregation rate of the clay. For kaolinite and phosphatic clay, the time spent in the first two zones ranged between 20 and 25 min. However, most of the bentonite mass remained in the first two zones up to 90 min, after which the massive agglomerate settled through the medium as one unit.

In Zone 3, the clay-cell aggregation is taking place. This is also the zone in which large aggregates pass through the water column via sweep floc process. Most of the cell removal is occuring with this portion, especially in phosphatic clay and bentonite, and the overall efficiency is determined by the effectiveness of the sweep-floc process.

In Zone 4, the falling aggregates accumulate and are lost to the system.

Modelling aggregation/settling and cell removal.

Using the conceptual model developed above, modelling focused on Zone 3 where clay-cell aggregation and settling was taking place, and the majority of cell removal was occurring. Based on these results, the most appropriate model was a first order equation:

$$\frac{dN}{dt} = -k\,N \qquad \text{(Eq. 5-1)}$$

where N = the total mass of particles (cells+clays) per unit volume and k is the rate constant which is determined by the physical and chemical properties of the system and the surrounding medium. The integrated form of the function is the following:

$$\ln N = N_o - kt \qquad \text{(Eq. 5-2)}$$

The model results, curve fits (least-squares fit) are reported in Table 5-3. The settling rates were calculated by multiplying the slope k (1/min) with the height of the water column (6.3 cm).

Discussion

Most of the previous work on HAB removal using clays have quantified overall cell removal by comparing on the endpoints of the process (i.e. initial versus final cell concentration over a prescribed time interval). However, these studies have only provided information for relative comparisons of the removal ability of clays without exploring the manner in which cell removal was taking place. Furthermore, earlier

attempts to study the process of clay-cell aggregation have focused on the physical and chemical properties of the system, using the information to assemble a working model of the process, but no work had been done to combine these properties into an actual, functioning system. In this study, the aggregation and settling was examined over time, in a dynamic process.

Calibration. The results showed that the concurrent use of the spectrophotometer and fluorometer were adequate and reliable in measuring the mass concentration of clays and cells simultaneously even when the two were mixed within therange of values used in this study. The slight variations in the data may be due to light scattering which interferred with the readings.

Kinetic experiments - Clays alone. The three clay minerals displayed rather different kinetics of aggregation and settling in the absence of algal cells, although the phosphatic clay and kaolinite were more similar to each other than to bentonite (Zone 1).

The kaolinite H-35 aggregated quickly and produced the first settling aggregates. Previous studies have shown that kaolinite suspensions aggregated faster than montmorillonites (Whitehouse et al., 1960; Postma, 1967; Hahn and Stumm, 1970). In a recent study (Chapter 4), the electrophoretic mobility (EPM) of this kaolinite in freshwater indicated an electronegative charge comparable to the bentonite, suggesting good stability in freshwater (Table 5-1). In seawater, this kaolinite showed the lowest EPM (i.e. most unstable), but the differences among the clays were slight. Therefore, it is unclear whether the stability of this clay, going from freshwater to seawater, is key to its fast aggregation rate. Another explanation may be that this clay has larger particles relative to the other two. The evidence for this may be found in the spectrophotometer calibration which showed a higher apparent absorbance at 500 nm for the same clay mass, relative to the other two clays (Figure 5-2). If such larger particles were present, it would affect the collision frequency between the particles, especially those due to differential sedimentation, leading to higher aggregation rates and a quicker transition to the settling phase.

The phosphatic clay was the second fastest clay to aggregate and settle. The low electrophoretic mobility of this clay in both freshwater and seawater, indicating relatively low stability, supported this observation more readily than in the kaolinite case. This low

EPM may be explained by the high Ca^{2+} concentration in the water used to produce the phosphatic clay in Florida (Bromwell, 1982). Compared to kaolinite, the aggregates leaving the turbid layer appeared larger. The larger flocs may contribute to higher mass transport over the same time interval compared to kaolinite (Figure 5-5). Furthermore, these larger agglomerates may sweep a column of water with a larger cross-sectional area, leading to better cell removal. This clay yielded higher *K. brevis* removal relative to the kaolinite by a factor of two.

The bentonite displayed the slowest aggregation rate. This presumably reflects the high stability of the clay suspension in freshwater, and the highest relative stability in seawater (Table 5-1). Olphen (1963) proposed this phenomenon was due to the higher stability of the electrical double layer of montmorillonite and bentonites over that of kaolinites. Flocs were observed near the freshwater-seawater interface early in the experiment (25 min), but very few were observed to settle, suggesting that their density was low (i.e. not much different from the surrounding seawater). This may be explained by the high apparent porosity of the flocs, as the material appeared voluminous, fluffy and delicate. Finally, settling occurred near the end of the experiment when the aggregates reached the appropriate size, which was essentially the thickness of the entire turbid layer itself. This falling aggregate swept a column of water with a diameter equal to the inner diameter of the test tube.

Kinetic experiments - Clays with *Karenia brevis*. Focusing on the clay behavior with added cells, the curves (i.e. aggregation and settling patterns) were generally similar to the case when cells were absent (Figure 5-6). However, the onset of settling appeared to be delayed for phosphatic clay and kaolinite. According to Stoke's Law, the settling rate is proportional to two factors (Stumm and Morgan, 1996): (1) the square of particle size, and (2) the density difference between the particles (or aggregates) and the surrounding medium. If the clays were co-aggregating with the cells, especially near the bottom of the turbid layer in Zone 1, the aggregate size would be expected to increase dramatically since *K. brevis* is much larger (length = 23 μm, breadth = 26 μm, thickness, 12.7 μm) than the initial clay particles. If this were the case, then the settling rate would increase. Alternatively, a reduction in settling speed may be achieved if the aggregate density decreases relative to the density of the primary particles (i.e. the clays). Clay

minerals typically have an average specific gravity of 2.6. Conceptually, the incorporation of algal cells into the aggregates will cause a decrease in particle density since algal cells have densities only slightly higher than seawater (Walsby and Reynolds, 1980). *Karenia brevis* has a density of 1.07 g cm^{-3}. As aggregation continues, the increase in particle size can overcome the decrease in aggregate density, leading to settling. This scenario may explain the results with phosphatic clay and kaolinite (Fig. 5-6A and C). As for the bentonite, it did not display a pronounced delay in the onset of settling. However, it appeared that the presence of cells in the floc promoted the layer to settle sooner (Figure 5-6B).

The critical question in this study is how cell removal efficiency is associated with the kinetics of aggregation and settling in the presence of clay minerals (Zone 3). In the case of phosphatic clay and algae, the clay appeared to be removing cells at the same time they were aggregating and settling (Figure 5-7A). Despite the initial delay in the start of settling, relative to clays alone (Figure 5-6A), the kinetics of the process proceeded in the same manner. The same scenario appears to be taking place for the kaolinite, at least during the first 40 min (Figure 5-7C). The trends of clay and cell loss in kinetic data and were parallel. Then, the clay continued to aggregate and settle as in the case of clays alone, however, cells were not being removed at the same rate as clay sedimented out. One explanation may be the ability of the organisms to free themselves from the sinking aggregates, once they reach a certain size. This effect may be possible if the attachment of the clay and clay is not as strong as with this organism and other clays. Other workers have also found poor removal of algae with kaolinite (e.g. Soballe and Threlkeld, 1988; Shirota, 1989).

Lastly, the removal ability of the bentonite did not seem consistent with its aggregation rate. Cell removal in this case may be achieved when a few large clay aggregates successfully leave the turbid layer and sweep the water column during descent. The particles were more effective than phosphatic clay even though they were not as abundant because the bentonite flocs were so voluminous. Eventually, the remaining cells in the medium were captured by the massive bentonite floc that began to sink through Zone 3 after 90 min. Therefore, the slow initial aggregation rate of this clay did not necessarily equate to poor cell removal, and vice versa (in the case of kaolinite).

Moreover, this system suggested that cell removal may not be just a simple co-aggregation phenomenon with clay particles as suggested in most of the current the literature. Cell removal may occur by particle interception and filtration (Shirota, 1989; Sengco et al., 2001).

In summary, IMC-P2 phosphatic clay aggregated quickly and incorporated cells into the flocs well. The larger, sinking agglomerates may continue accumulating cells as they settled. By contrast, WB-B bentonite aggregated very slowly but produced enough large aggregates throughout the experiment to continuously capture organisms during settling. Whatever cells that remained may be removed when the huge bentonite floc settled. Finally, H-35 kaolinite quickly aggregated similar to the phosphatic clay but cell removal trailed after a certain time point. The exact mechanism for this reduced removal is unknown but may be associated with the surface properties of the clay and cell, or the ability of the organism to escape the sinking flocs.

Modelling aggregation/settling and cell removal. The rate constant k (min^{-1}) for the three clays alone was consistent with the empirical observations: both kaolinite and phosphatic clay aggregated and transitioned to settling faster than the bentonite as reflected by their settling velocity (Table 5-3). The higher settling velocity for phosphatic clay compared to kaolinite may reflect the larger particle sizes produced by the phosphatic clay, thus leading to higher mass flux. When cells were added, the settling velocities for both kaolinite and phosphatic clay decreased by almost one-half, which agreed with observations and the hypothesis that the incorporation of buoyant cells into the aggregates may decrease the overall floc density, slowing settling rate. Conversely, the addition of cells appeared to enhance the deposition of the bentonite slightly and increase settling rates for the aggregates. Finally, the total mass flux (cells + clays) for phosphatic clay and bentonite were higher than the kaolinite which supported the observation that cells were no longer being transported by the kaolinite beyond a certain point in the process. Instead, the rate constant of kaolinite in the presence of *K. brevis* continued to decrease slightly and settling rate remained constant. By contrast, the value to bentonite increased steadily as cells were added to the floc mass.

The results of this analysis indicated that the removal of *K. brevis* with clays in this experimental system occurs not just with straightforward co-aggregation with fine

particles but with larger clay aggregates that collide by differential sedimentation and a sweep-floc process as well. Moreover, the different clay minerals have different means of capturing the organisms, though the final removal efficiencies were the same (e.g. for phosphatic clay and bentonite). Fast aggregation does not necessarily ensure high removal (e.g. kaolinite). However, the size of clay aggregates leaving the surface as well as the cohesiveness of these aggregates towards the algae may be important. For example, Shirota (1989) proposed that the higher absorptive (ion-exchange) capability of this three-layered clay mineral such as montmorillonite may be the reason for its effectiveness. In general, montmorillonites and deposits with a large proportion of montmorillonites showed better overall removal for many algae over kaolinites and zeolites (Maruyama et al., 1987; Sengco et al., 2001). More studies are needed to investigate the removal kinetics of other species (e.g. smaller cells, non-motile cells). Also, this system should studied when water motion is present.

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CHAPTER 6

Summary and Conclusions

This thesis demonstrated that domestic clays can effectively remove a number of bloom-forming species from the U.S., provided new insights into the aggregation and settling dynamics of the clay-algae system, and furnished information regarding the impacts of clay treatment on cell viability, growth, recovery and on the composition of the phytoplanktonic community due to selective removal of species.

Karenia brevis and clay minerals

In general, montmorillonite and deposits containing a large proportion of montmorillonite (i.e. phosphatic clays) have consistently shown high removal efficiency (RE) with relatively small amounts of material (< 0.04 g l^{-1}). RE increased with clay loading but reached a maximum value (> 90%) with as little as 0.25 g l^{-1}. Based on these laboratory experiments, the amount of highly effective clays needed to treat *K. brevis* are comparable to or less than the amount of clay used to treat HABs in Japan and South Korea. While clay treatment consistently produced much better results than alum, poly-aluminum chloride (PAC) and several flocculants by a factor of 2, the combination of phosphatic clay and a small quantity of PAC (5 mg l^{-1}) further enhanced the removal ability of this clay. This strategy may potentially lower the amount of clay needed to maintain high removal rates.

In later studies using phosphatic clay, the RE of *K. brevis* generally increased with cell concentration. However, when the cell concentration fell below 1000 cell ml^{-1}, RE also dropped significantly to below 70% even for clay loadings up to 0.50 g l^{-1}. This result suggested that a critical number of cells should be present in order for clay removal to achieve its full potential. In practice, such as threshold in cell concentration was also found in South Korea where clay dispersal was used only when *Cochlodinium* concentrations have exceeded 3000 cells ml^{-1} (H.G. Kim, personal communication). One explanation for this phenomenon may be that the presence of larger particles like algal cells in the growing aggregates are need to effectively remove other cells farther down the water column (i.e. via sweep-floc mechanism, Chapter 5). Conversely, aggregates consisting mostly of clay particles are less efficient at entraining cells from the water column by virtue of their smaller size. Unfortunately, these results also suggest that clay treatment may offer limited relief at low *K. brevis* concentrations where deleterious impacts can

already be detected but are too low to be affected by clays. Nevertheless, these findings can guide future applications with respect to the timing of clay dispersal and cell abundance during an outbreak, or with regards to the distribution of the organisms in the water column.

K. brevis remained viable and soon recovered when phosphatic clay loading fell below 0.03 g l^{-1}, with or without resuspension of the aggregated material. At 0.50 g l^{-1}, cell mortality approached 100% after 12 h, and no recovery was found even with daily resuspension. The mortality of *K. brevis* was associated with direct contact with clay minerals, and not by the release of cytotoxic substances from the clay, or from the lysed cells. However, the exact mechanism causing cell death is unknown. At intermediate clay loadings (e.g. 0.10 to 0.25 g l^{-1}), the survival and subsequent recovery of the algae depended on the clay loading, the frequency of resuspension, and the duration of contact between the clays and cells prior to the first resuspension event. Cell death increased dramatically after 12 h, but recovery rates improved if resuspension occurred before this time, and when resuspension occurred frequently.

While the use of smaller clay amounts to treat an outbreak is an important consideration in minimizing its potential impacts, these findings suggest that too little clay may allow the target organisms to escape, recover and re-establish themselves in the water column shortly after treatment. In addition, resuspension and turbulent forces that can allow for escape should also be factored in calculating the appropriate clay dosage and the timing of dispersal (i.e. tidal cycle). In summary, the highest *K. brevis* removal by clays can be achieved by considering both the cell concentration and the clay loading which will entrain the cells long enough for mortality to occur though direct contact with clay, removal from the photic zone, entrapment in the sediment, or any other mechanism.

Electrophoretic mobility of clay minerals

There were clear differences in the electrophoretic mobility (EPM) of the various clay minerals in freshwater suspensions. Montmorillonites (bentonites) consistently displayed the highest EPM, and therefore, they possessed highest stability among the clays in this medium. By contrast, the phosphatic clays have among the lowest EPM values which may be due to the large amount of Ca^{2+} in the medium. The kaolinites and zeolites

showed a range of intermediate values. Aggregation was not observed in the clay suspensions. In natural seawater (29.6 salinity), all of the EPM values were expectedly reduced and rapid aggregation took place. In addition, this reduction of EPM occurred even in very dilute seawater (1.8 salinity). Therefore, clay stability and aggregation are controlled by the double layer thickness and ionic strength. Based on these data there were no apparent electrophoretic differences among the various minerals in seawater to provide a direct explanation regarding the effectiveness of one clay mineral over another for a given algal species (e.g. *Karenia brevis*).

Kinetics of clay-algae aggregation, settling and cell removal

Kinetic studies using *Karenia brevis* and three clay minerals (phosphatic clay, montmorillonite and kaolinite) revealed different ways by which aggregation and settlement occurs, and how these processes were related to cell removal. The experimental system was designed to simulate the most likely dispersal scenario in the field: the clay slurry was added to the surface of the water column containing cells, forming a turbid layer. At the interface between the seawater and freshwater, the aggregation process produced flocs (i.e. first phase), which then settled through the medium and interacted with the organisms (i.e. second phase). The same initial cell and fractionated clay concentrations were used.

In the case of phosphatic clay and kaolinite, aggregation occurred quickly in the absence of *K. brevis*, and aggregate settling was observed within 20 min and continued for another 40-50 min. For phosphatic clay, this behavior is due to its low stability (low EPM) even in freshwater. For kaolinite, its stability was higher than the phosphatic clay in freshwater but it quickly dropped in seawater. In addition, this clay may consist of slightly larger particles than those found in the other clays. Bentonite aggregated more slowly by virtue of its high stability (high EPM) in freshwater and its relatively high stability in seawater as well. After 90 min, half of the initial clay mass settled as one massive aggregate that swept through the water column.

In the presence of *K. brevis*, all of the clays maintained the same kinetic patterns. However, the onset of settling was delayed by 5-10 min for phosphatic clay and kaolinite. This effect may be due to a reduction in aggregate density from the incorporation of cells

into the floc (low density particles which may buoy the floc). After settling began, the phosphatic clay and kaolinite followed the same kinetics. For phosphatic clay, cell removal strongly coincided with the settling phase of the clay, leading to 87% removal over 60 min. For the kaolinite, the same pattern was emerging, but after 40 min, the rate of cell removal decreased markedly while the aggregation and settling of kaolinite particles continued unabated. Cell removal only reached 31% after 40 min and achieved 46% at the end of the experiment (150 min). With bentonite, the aggregation rate of clay was limiting, but a few voluminous flocs were produced over 90 min. Cell removal also proceeded slowly but steadily. The RE at 90 min was 72% but increased to 97% after the massive floc swept the column. These observations suggested that the cell removal was dependent on the particle concentration in all three cases (first order process).

These results demonstrated the different behaviors of clay aggregation and settlement, and how they can affect cell removal. The initial rapid aggregation and transition to settling by kaolinite did not ensure a high cell removal as it did for phosphatic clay. By the same token, bentonite accomplished exceptional cell removal though it aggregated more slowly. One key difference may be in the size of the aggregate produced by the clay layer which falls through the medium. Other indications suggest a poor association between *Karenia brevis* and kaolinite, although the nature of this association remains unclear (e.g. low stickiness, ability of the cell to escape).

These results also showed that clay-cell aggregation is also occurring near the surface, and the presence of cells in the floc can affect both the size and density of the aggregate. This may explain the earlier finding that a critical number of *K. brevis* must be present for clays to reach their highest values. It is possible that the nature of these first few clay-cell aggregates can influence the removal of other cells farther below in the water column. Finally, the study of clay-cell kinetics revealed information about the system that was not readily apparent just by analyzing the physical and chemical properties of the particles (e.g. EPM). In these experiments, the varying stabilities of the clay clearly played an important role in their kinetics. These differences were more long-lived than expected probably due to the use of freshwater to prepare the slurry, and the initial density-driven separation between the freshwater and seawater.

Physical and charge properties of marine microalgae, and their removal with clay

In this research, phosphatic clay displayed a range of removal abilities against a variety of algal species. While the RE for each organism increased with clay loading (up to 0.50 g l^{-1}), the trends were not as clear with increasing cell concentration. For nine out of seventeen species, RE increased with increasing cell number. These include *Karenia brevis*, *Akashiwo sanguinea* (formerly *Gymnodinium sanguineum*), *Heterosigma akashiwo* and *Heterocapsa triquetra*. In the remaining eight, RE either increased initially then decreased, or remained low and constant.

When the similar species were grouped and compared at relatively similar cell numbers, the RE did not correlate well with the projected cross-sectional area of the cells, their swimming speeds, or a certain type of surface covering (i.e. theca, silica frustule). For the flagellated species, the total collision frequency coefficents were calculated for all the possible collision mechanisms (including cell motility). These values showed a better correspondence to the empirical removal efficiency. These results showed that collisions due to swimming may be an important transport mechanism, especially when cell sizes are less than 50 μm. In addition, size (as cross-sectional area) cannot be used as a means of predicting how well a certain clay will remove a given species. For *Karenia brevis*, the high RE may be associated with this organisms size as well as its high swimming velocity.

By extending these results, some predictions can be made regarding the potential effect of clay treatment on other species. For example, bacteria and virus particles would not be removed by clays due to their size, similar to the findings with *Synechococcus* WH8017 and *Aureococcus anophagefferens*. Larger phytoplanktonic cells, especially the highly motile species, or groups of cells (e.g. chain forming species) would be removed most efficiently. However, strong cell motility can also lead to low removal when vigorous swimming can also allow the organisms to escape the floc following contact. As for the zooplanktonic species, strong swimming may prevent them from being captured by the clay particles.

This thesis presented the first known measurements of electrophoretic mobility (EPM) for marine microalgae. The data confirmed the assumption that marine species carry an electronegative charges, although much smaller in magnitude than those from

freshwater species. The range of EPM values and zeta potentials (ζ) indicated that the suspension of organisms was unstable yet aggregation was not observed in the cultures. These results suggest that the stability of cell suspensions in marine waters are controlled by the presence of organic molecules on the cell suface (i.e. steric stabilization), and not by salt destabilization or double layer thickness. These finding highlighted a possible means by which some marine phytoplankton, such as dinoflagellates, can minimize their stickiness and their propensity for aggregation, thereby limiting their possible loss to sinking. Moreover, low stickiness by steric stabilization, coupled with cell motility, may allow members of this group to attain high cell abundances while avoiding loss through massive aggregation.

A comparison of the EPM values and ζ for various species to their empirical RE did not show a pattern. Despite this, there is much empirical evidence from other studies in this thesis suggesting that differences exist in the affinity of organisms for a given clay (see above). It is possible that the level of specificity between clays and the cell surface features lie in the quality or type of the organic molecules. Finally, some biological property may explain the removal patterns of organisms with phosphatic clay (i.e. positive or negative taxis, the release of mucous on contact, ecdysis).

Selective removal of algal species by phosphatic clay

This research demonstrated that clay treatment (using phosphatic clay) did not remove all species indiscriminately. In fact, phosphatic clay displayed a wide range of removal values for different phytoplankton.

In laboratory experiments, *Karenia brevis* was removed preferentially over the dinoflagellate *Prorocentrum micans*, and the diatom *Skeletonema costatum* in mixed cultures. The RE of each organism in the combination was similar to or slightly better than their RE for each cell alone, especially in *K. brevis*. The same observation was made during a mesocosm experiment where a natural assemblage during a *K. brevis* bloom was treated with phosphatic clay. While the RE of *K. brevis* was not exceptionally high, the value (49% at 0.05 g l^{-1} clay) was much higher than would be expected (based on laboratory trials) given the low initial concentration of cells in the bloom (< 200 cells ml^{-1}). The two dominant diatoms *Skeletonema* sp. (55.2% RE) and *Bacillaria* sp.

(34.1%) were also removed by phosphatic clay. However, the minute species such as *Prymnesium* sp. was relatively unaffected by clay treatment, although the high variability in the sample made this result tentative. Nevertheless, these have been the first known studies where the removal of multiple species in the same suspension were monitored during clay treatment.

These results suggested that clay removal can selectively remove a target species like *K. brevis* with the appropriate clay. In mesocosms, clay treatment did not turn the water column into a cell-free desert as other expected. Moreover, the high effectiveness of clay can still be maintained when the concentration of the target species is low if other species are present in the water column. Only a slight removal of these co-occurring algae may be needed to "replace" the missing "biomass" of the target cells in order to affect their removal.

Future directions

The results of this research provide new insights into the aggregation of minerals and living cellular particles. Such aggregates, called marine snow, form naturally and have significant biological, ecological and geochemical significance in marine waters. The information developed in this work may contribute to the understanding of the formation and dynamics of these particles.

Additional studies will be needed to further elucidate the surface characteristics of marine phytoplankton that influence their removal efficiency with clay. A biological or biochemical explanation may yet be found. For example, simple biochemical assays can be performed to identify the molecules on the cell surface (e.g. carbohydrates, proteins). Video microscopic observations can be used to determine whether cells are attracted or repulsed by clay, and whether cells can escape from the floc after capture.

Studies on the kinetics of aggregation and settling have revealed different behaviors among clays and their removal of cells. Simple models focusing on the mass flux have been developed in this research. Other models using particle size distribution have proven useful in understanding aggregation and settling. However, sampling methods or non-invasive observational systems need to be developed in order to obtain size information for such models. In addition, removal experiments should also be

conducted in flowing water. Laminar or turbulent flow can influence collision rates as well as particle breakage. Water flow can affect the ultimate size, shape and strength of the aggregate.

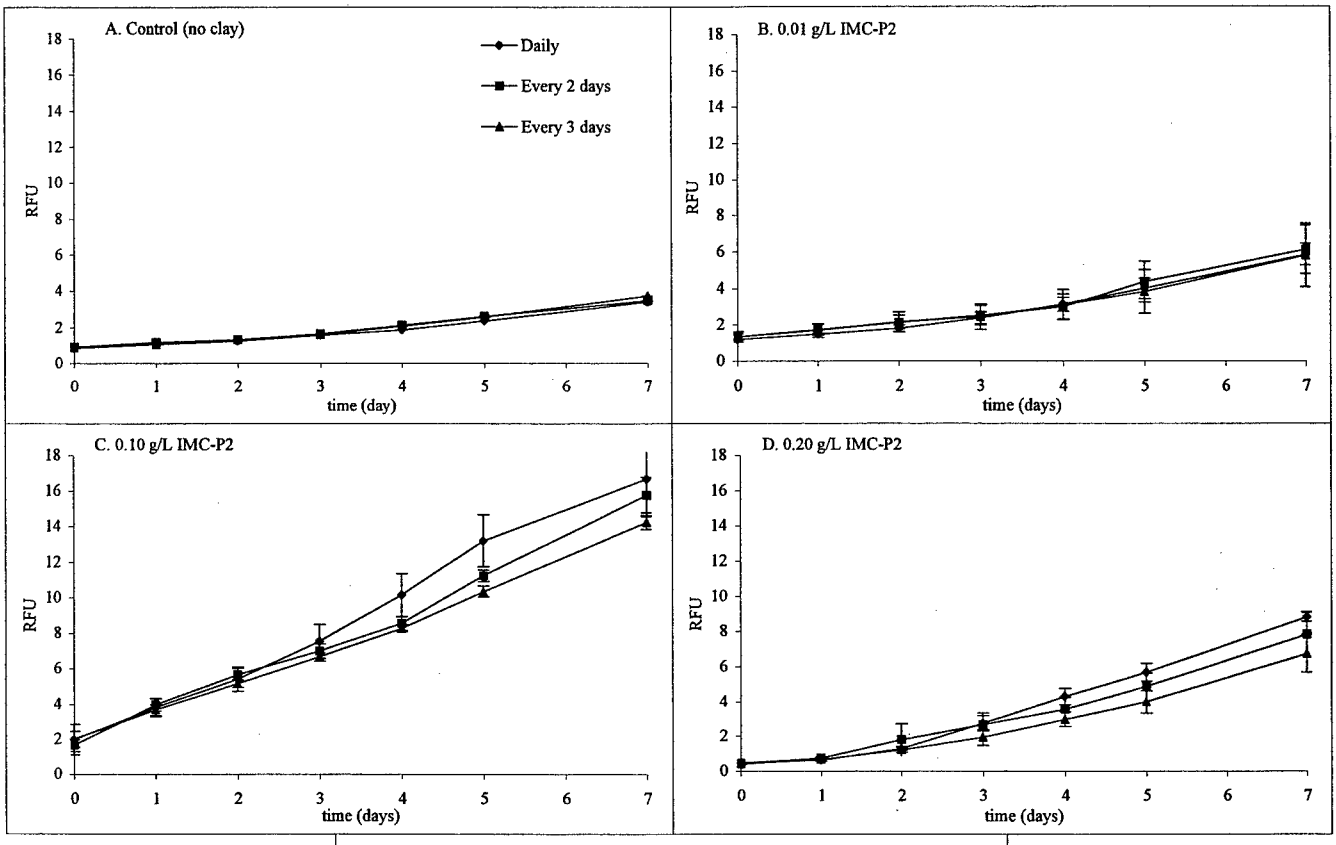
The consequences of selective (or differential) removal of species must be addressed in long-term studies. It could be hypothesized that the composition of the community will change in the short term, leading to the removal of species with high affinity to clay and the enrichment of resistant forms. However, the long-term outcome may depend on a large number of factors such as cell recovery, escape from the floc, and the biological/physiological properties of the organisms. This will be an important area of investigation.

The impact of clay addition on the phytoplanktonic community may also be indirect, particularly though light attenuation, the release and/or adsorption of algal nutrients, and the alteration in grazing pressures. Although aggregation and settling proceed rapidly in most of the clays studied, some fine clay particles remain in the water column hours after dispersal based on direct observations. These particles may decrease light penetration and hinder primary production. Inorganic and organic nutrients may be released by the clays, leading to a stimulation in primary production, although the effect may be moderated by the availability of other nutrients in the water column or the degree of light attenuation due to increased turbidity. Conversely, the adsoprtion of certain algal nutrients can inhibit growth and primary production. Finally, the presence of clay minerals or residual turbidity may affect the ability of grazers to consume algal prey by interfering with their feeding behavior. For example, filter feeders that remove particles indiscriminately may be affected more by the higher number of inedible particles in the water column compared to selective feeders that choose their food items. More studies will be needed using natural assemblages and entire communities of organisms in order to undertand the possible impacts of clay on the ecology of the water column.

Appendix

The figures in this section represent the data that were not shown in Chapter 2 which was published in Marine Ecology Progress Series, and in Chapter 3 of this thesis.

Figure A-1. Recovery of *Gymnodinium breve* following clay treatment (IMC-P2 phosphatic clay) with resuspension. Culture fluorescence (in relative fluorescence units = RFU) over time (in days), with the first measurement beginning after the 2.5-hour removal experiment. Clay treatments include (A) no clay, (B) 0.01 g l^{-1}, (C) 0.10 g l^{-1}, (D) 0.20 g l^{-1}, and (E) 0.50 g l^{-1}. Each graph compares cell recovery at the same clay loading but with varying resuspension frequency: daily, every 2 days, every 3 days. Error bars represent standard deviation (n = 3).


A. Control (no clay)
Daily
Every 2 days
Every 3 days
RFU
time (day)
B. 0.01 g/L IMC-P2
RFU
time (days)
C. 0.10 g/L IMC-P2
RFU
time (days)
D. 0.20 g/L IMC-P2
RFU
time (days)

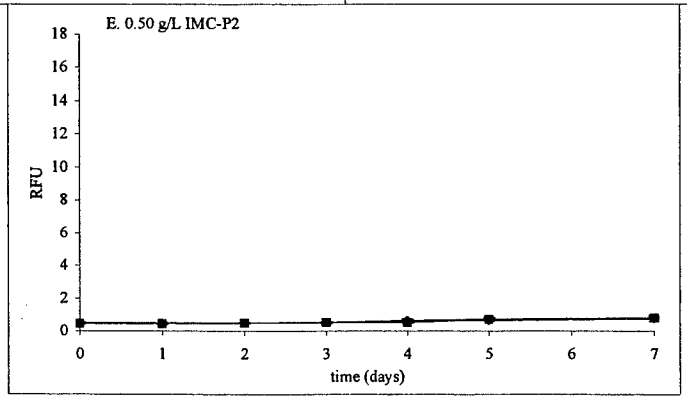

E. 0.50 g/L IMC-P2
RFU
time (days)

Figure A-2. Removal efficiency of *Aureococcus anophagefferens* using zeolites: SW-ZP (Southwestern zeolite powder), SW-NM (Southwestern Nicole Mountain), and SW-NZ (Southwestern Natur-Zeo).

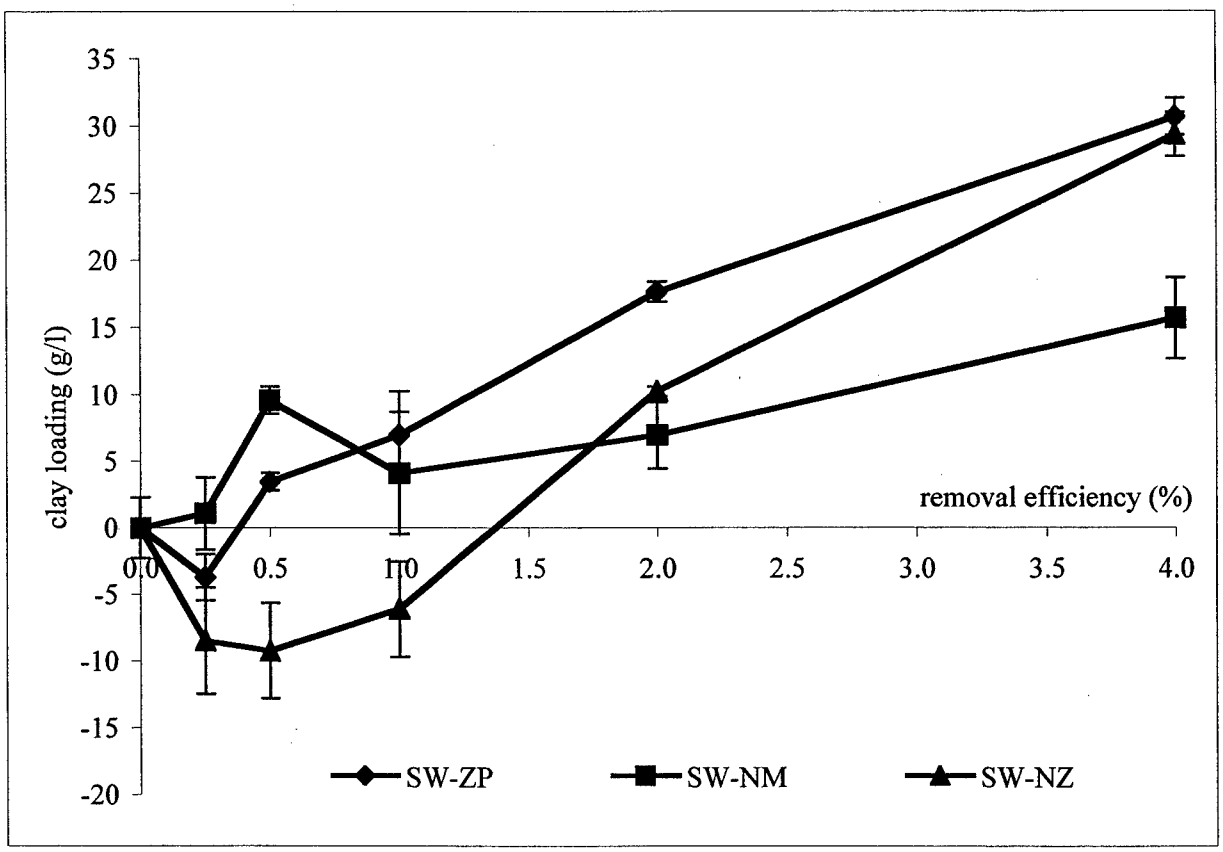

35
30
25
20
15
10
5
0
-5
-10
-15
-20
clay loading (g/l)
removal efficiency (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
SW-ZP
SW-NM
SW-NZ

Figure A-3. Removal of Aureococcus anophagefferens using H-DP (Huber treated kaolinite) and IMC-P2 (phosphatic clay) treated with polyaluminum chloride, Percol 778 (cationic polymer) and Percol 720 (nonionic polymer).

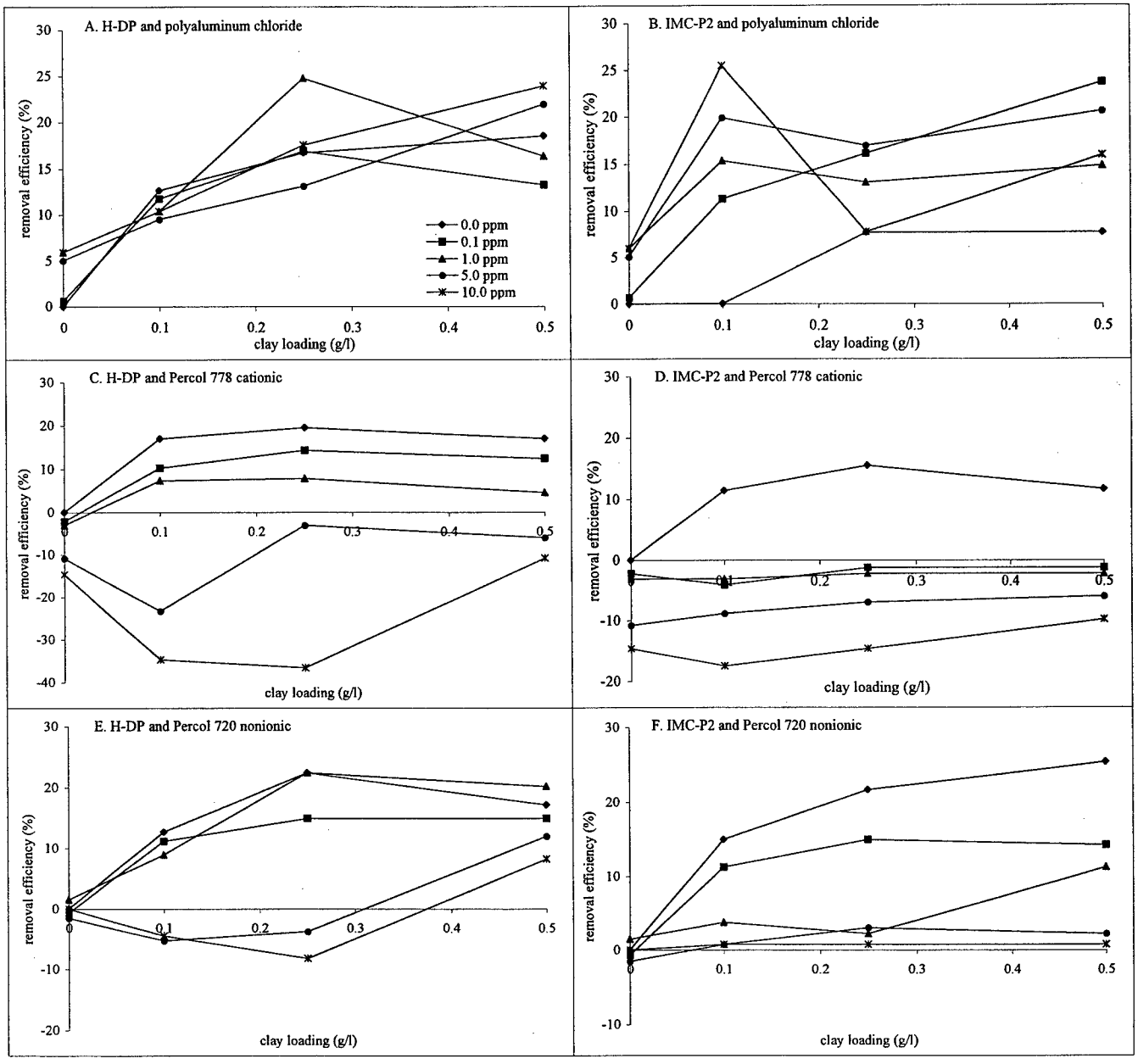

A. H-DP and polyaluminum chloride
B. IMC-P2 and polyaluminum chloride
C. H-DP and Percol 778 cationic
D. IMC-P2 and Percol 778 cationic
E. H-DP and Percol 720 nonionic
F. IMC-P2 and Percol 720 nonionic
removal efficiency (%)
clay loading (g/l)
0.0 ppm
0.1 ppm
1.0 ppm
5.0 ppm
10.0 ppm

Figure A-4. Comparison of removal efficiency with geometric characteristics of algal species studied. (A) Cell length, (B) Cell breadth, (C) Cell thickness. Total collision frequency coefficient of flagellates at clay size = 10 μm. The algal sizes used in the calculation are listed in Table 3-2. The linear regression was plotted along with the R-squared value.

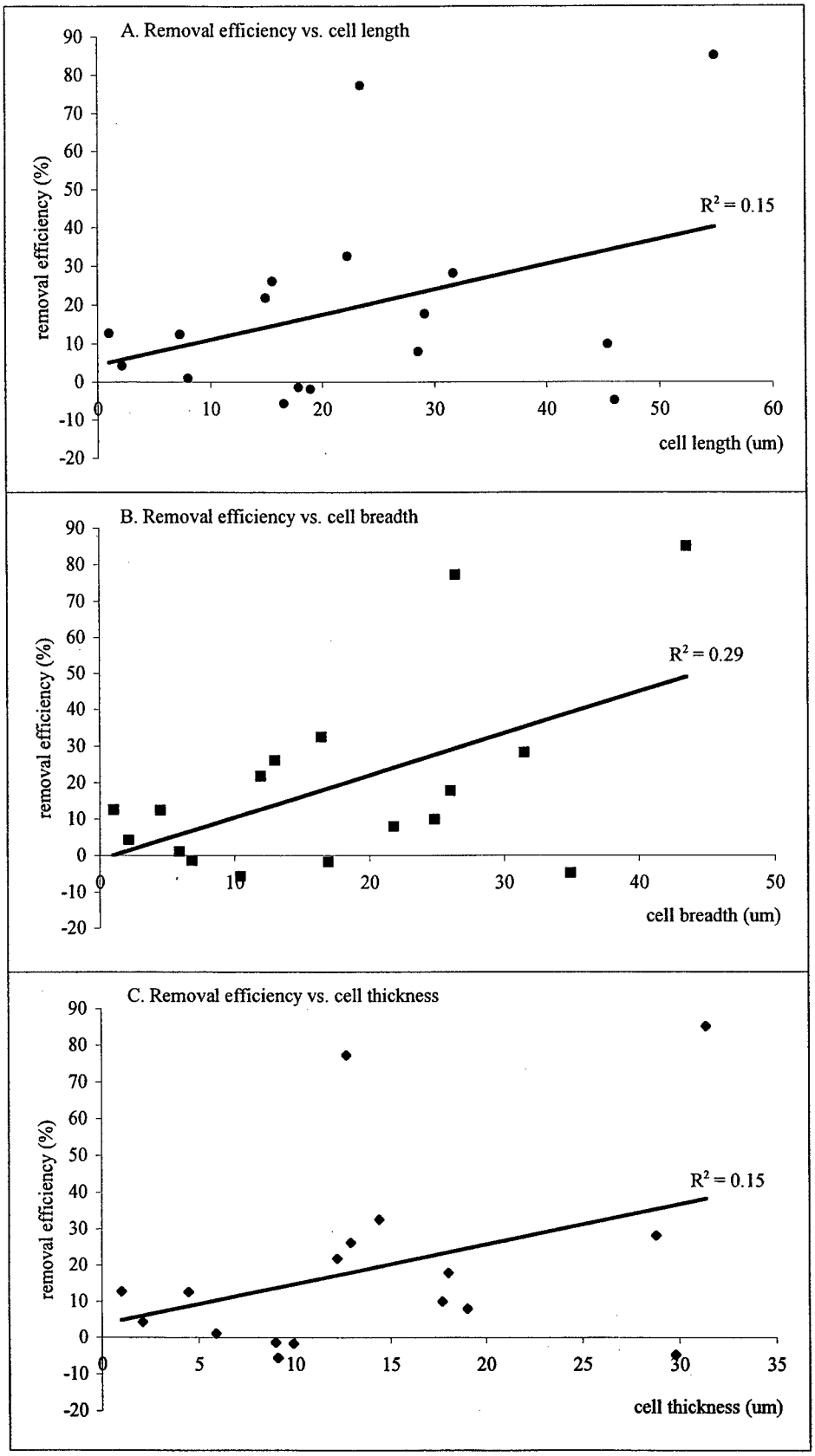

A. Removal efficiency vs. cell length
removal efficiency (%)
cell length (um)
R² = 0.15
B. Removal efficiency vs. cell breadth
removal efficiency (%)
cell breadth (um)
R² = 0.29
C. Removal efficiency vs. cell thickness
removal efficiency (%)
cell thickness (um)
R² = 0.15

Figure A-5. Collision frequency coefficients for *Karenia brevis*. Brownian diffusion, swimming motility, differential sedimentation and fluid motion. Shear rates were 0.30 s^{-1}, 3.0 s^{-1} and 30 s^{-1}. The equations and variables are listed in Table 3-1.

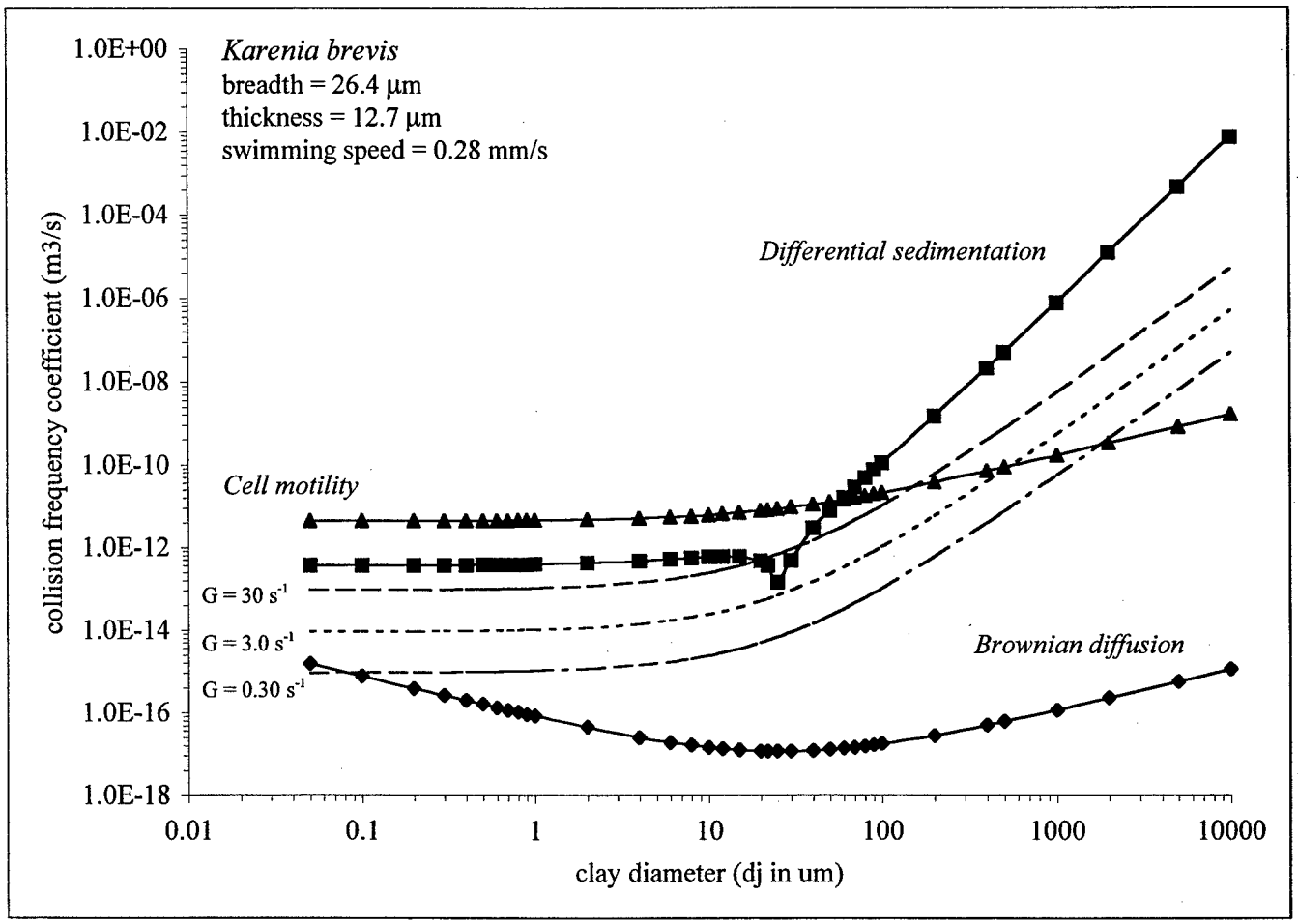
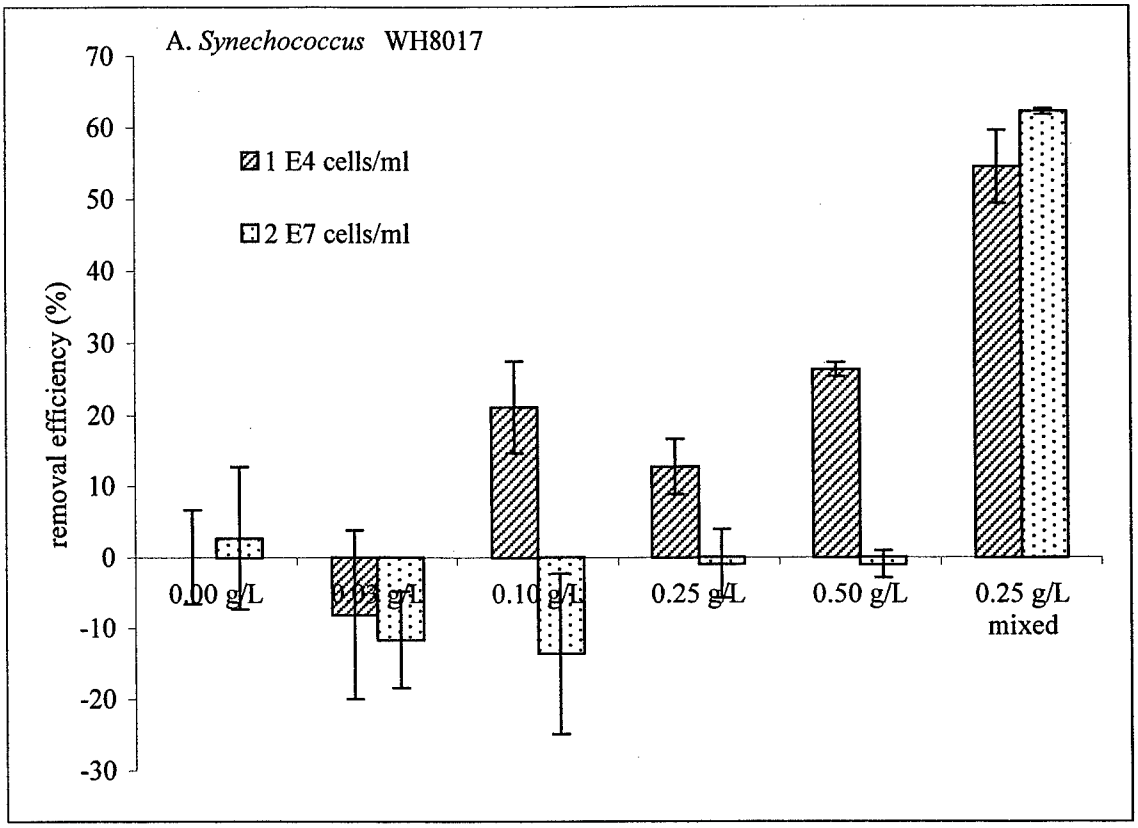

Karenia brevis
breadth = 26.4 µm
thickness = 12.7 µm
swimming speed = 0.28 mm/s
Differential sedimentation
Cell motility
G = 30 s-1
G = 3.0 s-1
G = 0.30 s-1
Brownian diffusion
collision frequency coefficient (m3/s)
1.0E+00
1.0E-02
1.0E-04
1.0E-06
1.0E-08
1.0E-10
1.0E-12
1.0E-14
1.0E-16
1.0E-18
0.01
0.1
1
10
100
1000
10000
clay diameter (dj in um)

Figure A-6. Removal efficiency of (A) *Synechococcus* WH8017 and (B) *Aureococcus anophagefferens* with IMC-P2 phosphatic clay, with and without mixing.


A. Synechococcus WH8017
1 E4 cells/ml
2 E7 cells/ml
removal efficiency (%)
70
60
50
40
30
20
10
0
-10
-20
-30
0.00 g/L
0.03 g/L
0.10 g/L
0.25 g/L
0.50 g/L
0.25 g/L mixed

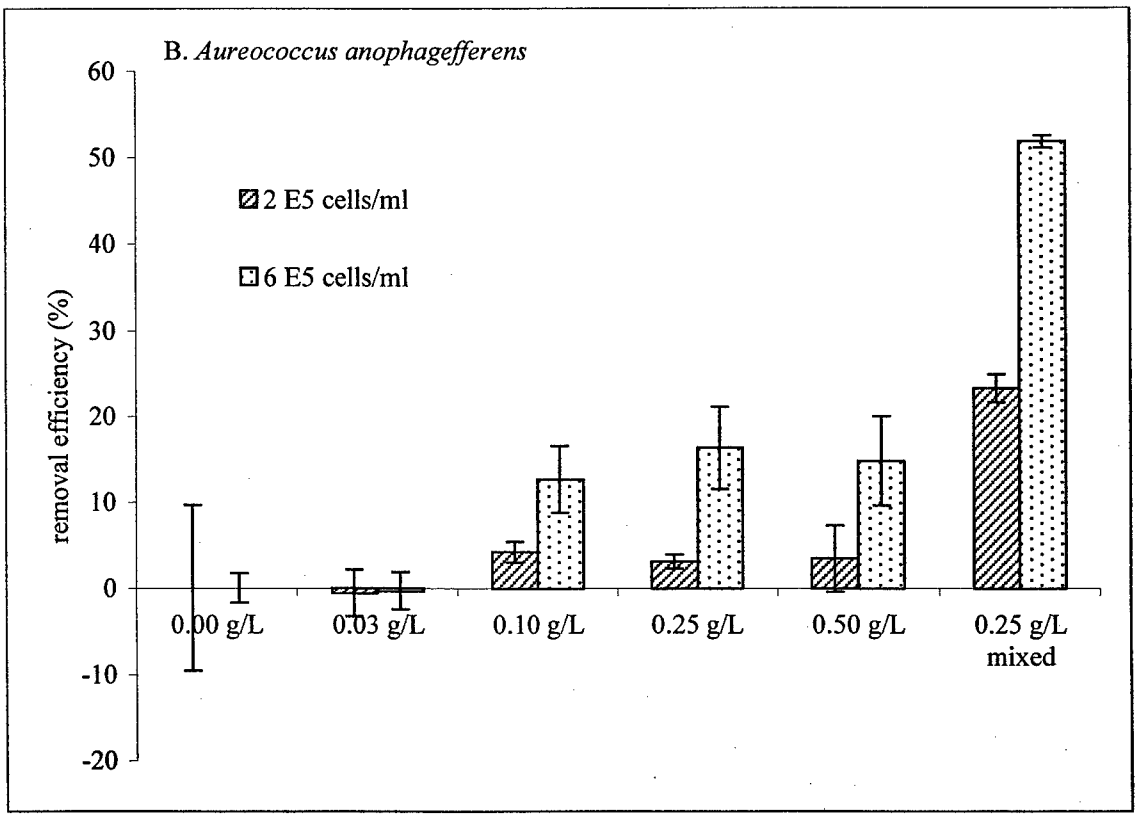
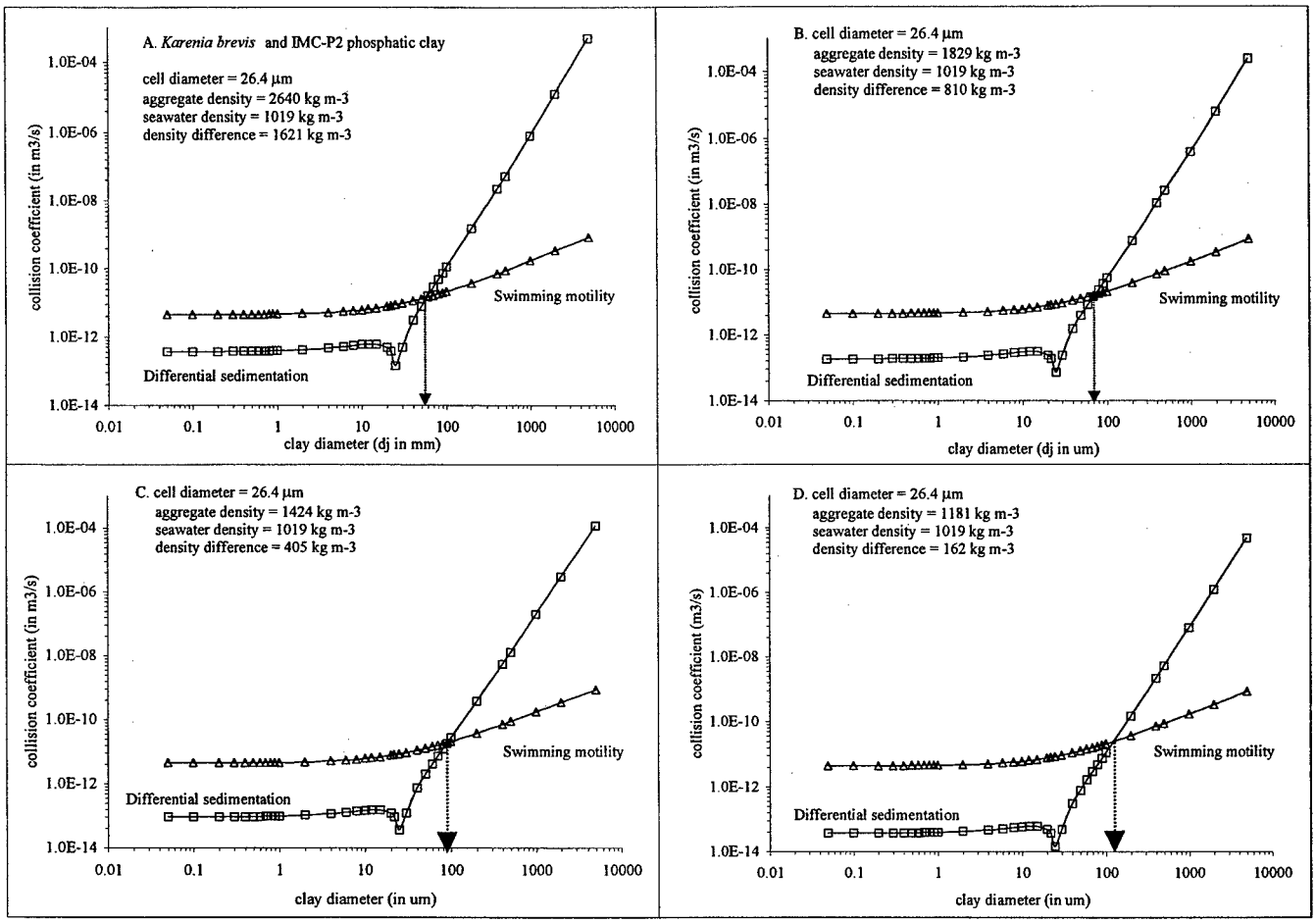

B. Aureococcus anophagefferens
2 E5 cells/ml
6 E5 cells/ml
removal efficiency (%)
60
50
40
30
20
10
0
-10
-20
0.00 g/L
0.03 g/L
0.10 g/L
0.25 g/L
0.50 g/L
0.25 g/L mixed

Figure A-7. *Karenia brevis* and phosphatic clay. Comparison of collision frequency coefficients for cell motility and differential sedimentation (equations and constants in Table 2-1). In each panel, the values for cell motility remain constant, while the values for differential sedimentation have been re-calculated to account for the possible reduction in aggregate density as aggregate size increases. (A) aggregate density = 2640 kg m^{-3} (initial conditions in Chapter 2), density difference between particle and seawater = 1621 kg m^{-3}. (B) aggregate density = 1829 kg m^{-3}, density difference = 810 kg m^{-3}. (C) aggregate density = 1424 kg m^{-3}, density difference = 405 kg m^{-3}. (D) aggregate density = 1181 kg m^{-3}, density difference = 162 kg m^{-3}. All other parameter remained constant.


A. Karenia brevis and IMC-P2 phosphatic clay
cell diameter = 26.4 µm
aggregate density = 2640 kg m-3
seawater density = 1019 kg m-3
density difference = 1621 kg m-3
Swimming motility
Differential sedimentation
collision coefficient (in m3/s)
clay diameter (dj in mm)
B. cell diameter = 26.4 µm
aggregate density = 1829 kg m-3
seawater density = 1019 kg m-3
density difference = 810 kg m-3
Swimming motility
Differential sedimentation
collision coefficient (in m3/s)
clay diameter (dj in um)
C. cell diameter = 26.4 µm
aggregate density = 1424 kg m-3
seawater density = 1019 kg m-3
density difference = 405 kg m-3
Swimming motility
Differential sedimentation
collision coefficient (in m3/s)
clay diameter (in um)
D. cell diameter = 26.4 µm
aggregate density = 1181 kg m-3
seawater density = 1019 kg m-3
density difference = 162 kg m-3
Swimming motility
Differential sedimentation
collision coefficient (m3/s)
clay diameter (in um)

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16. Abstract (Limit: 200 words)

Clay dispersal is one of the most promising strategies for controlling harmful algal blooms. It is based on the mutual aggregation of algal cells with mineral particles, leading to aggregate settling. This research demonstrated the effectiveness of domestic clays against bloom-forming species from the United States (> 90% removal efficiency, RE), such as *Karenia brevis* and *Heterosigma akashiwo*, at clay loadings ≤ 0.25 g l^{-1}. Algal viability and recovery depended on loading, resuspension frequency, and contact duration between clay and cells before the first resuspension event.

Phosphatic clay showed varying RE against seventeen species from five algal classes, and removal trends varied with increasing cell concentration. RE was not correlated with algal size, swimming rate, and the type of cell covering. RE was correlated with the predicted collision frequency coefficient. Selective removal of *K. brevis* by phosphatic clay was observed in mixed laboratory cultures, and in mesocosms containing a natural field assemblage.

Marine microalgae and clay minerals in natural seawater displayed a narrow range of negative electrophoretic mobilities (EPM). EPM values did not correlate with observed removal patterns. Kinetic studies of the clay-cell system revealed varying rates of aggregation and settling among bentonite, kaolinite and phosphatic clay with *K. brevis*

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